Original Article

EHD2 inhibits the invasive ability of lung adenocarcinoma and improves the prognosis of patients

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Background: EH domain contains protein 2 (*EHD2*) may be involved in tumorigenesis and development. However, the role of *EHD2* in lung adenocarcinoma (LUAD) is unknown.

Methods: The link between *EHD2* and LUAD and the associated underlying mechanism was determined using bioinformatics analysis. Then, immunohistochemistry (IHC) was employed to detect *EHD2* expression level in LUAD patients. The stable transfection cell line was used to establish with lentivirus vector, and then the transfection efficiency was detected by western blot. Phagokinetic motility assays, transwell assays, and western blotting were also employed to investigate *EHD2* impacts on cell viability.

Results: The results indicated that *EHD2* protein expression in human LUAD samples was significantly lower than that in the adjacent normal tissues. Low *EHD2* expression was significantly linked to lymph node metastasis as well as advanced tumor-node-metastasis (TNM) staging ($P<0.05$). The Kaplan-Meier survival curve showed that low *EHD2* expression was significantly associated with low survival ($P=0.01$). The multivariate Cox regression analysis confirmed that *EHD2* expression and TNM stage were independent prognostic factors for LUAD patients (all $P<0.05$). The in vitro experiments demonstrated that EHD2 knockdown markedly contributed to an increase in migration and invasion in A549 cells. Overexpression of *EHD2* substantially suppressed H1299 cell migration and invasion. Furthermore, decreased E-cadherin expression was observed in A549 cells with *EHD2* knockdown, as well as increased N-cadherin and vimentin expressions. By contrast, E-cadherin expression was increased in H1299 cells, whereas N-cadherin and vimentin expressions were decreased as a result of *EHD2* overexpression.

Conclusions: Our study demonstrated that *EHD2* reduces LUAD migration and invasion by preventing the epithelial-mesenchymal transition (EMT) process. Furthermore, the results suggest that *EHD2* may be a novel biomarker for prognosis prediction.

Keywords: Lung adenocarcinoma; *EHD2*; epithelial-mesenchymal transition (EMT); overall survival (OS)

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Introduction

Lung cancer is the most lethal malignant tumor worldwide with the highest mortality rate (1). Non-small cell lung cancer (NSCLC) accounts for approximately 80–85% of lung cancers, and lung adenocarcinoma (LUAD) has become the most prevalent subtype (2). At present, radiotherapy is the most important non-surgical treatment for LUAD (3). Although radiotherapy technology has advanced significantly, the survival rate has plateaued (4). Unfortunately, radiotherapy resistance is one of the main reasons for the failure of LUAD treatment, and its molecular mechanism has not been fully elucidated.

The curative effect of radiotherapy is affected by factors such as epithelial-mesenchymal transition (EMT), tumor inherent radiation resistance, and the tumor microenvironment (TME) (5-7). E-cadherin forms vesicles and regulates adhesion junctions through endocytosis, and the down-regulation of E-cadherin expression is the key event of EMT (8,9). Meanwhile, specific modules such as the EH (Eps15 homology) domain regulate the entire endocytosis process (10). The EH domain contains protein 2 (*EHD2*), a member of EHD protein family, which is critical for nucleotide-dependent membrane remodeling and is involved in membrane transport between cell and plasma membranes (11,12). *EHD2* is a mechanotransducing ATPase localized in caveolae invaginations at the plasma membrane. Its overexpression leads to suppression of internalization, and it may inhibit the migration and invasion of liver cancer by interacting with E-cadherin to improve prognosis (13). Research has shown that patients with colorectal cancer (CRC) who have *EHD2* up-regulation experience longer overall survival (OS). Overexpressing *EHD2* was shown to inhibit CRC cell migration and to increase the invasion, apoptosis, and cell cycle arrest (14,15). In addition, *EHD2* has been implicated in glioma and esophageal squamous cell cancer as a tumor suppressor gene (16,17); these studies showed that *EHD2* may be involved in tumorigenesis and development. Nonetheless, the role of *EHD2* in LUAD is unknown.

In the present study, using bioinformatics analysis, we determined that *EHD2* is critical in LUAD. Further experiments have shown that *EHD2* can suppress LUAD migration and invasion by preventing EMT process. In addition, we analyzed the association between *EHD2* level and prognosis in LUAD, suggesting that *EHD2* may become a biomarker for predicting LUAD prognosis. We present the following article in accordance with the TRIPOD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-842/rc).

Methods

Data acquisition

We obtained a dataset (535 LUAD and 59 normal samples) from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) LUAD database. Meanwhile, 170 LUAD tissues were provided by the Affiliated Tumor Hospital of Nantong University in China. From 2012 to 2013, all patients received surgical resection. The average follow-up time was 70 months. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the Affiliated Tumor Hospital of Nantong University (No. 2016-094). All participants provided written informed consent.

Biological information analysis

To detect *EHD2* expression in LUAD tissues, we utilized the Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Interactive Analysis (GEPIA) to construct box plots. At the same time, GEPIA was employed for analyzing the link between *EHD2* and E-Cadherin. The correlation coefficient was determined by the Spearman method. Kaplan-Meier Plotter was deployed for determining the link between *EHD2* and OS. Further, R software (The R Foundation for Statistical Computing, Vienna, Austria) was used to perform correlation testing on genes to find genes that have a co-expression relationship with the target gene. The selection conditions were ($|cor|>0.4$ & $P<0.05$). We found genes that have a co-expression relationship with the target gene, and used the pheatmap package to draw heat maps for the 20 most significant positive and negative correlations. We utilized the clusterProfiler package to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on differential genes ($P<0.05$), producing a bubble chart.

Gene set enrichment analysis (GSEA)

GSEA was undertaken to identify genes with significant differences based on the expression of *EHD2*. The arrangement was set to 1,000. We then performed

evaluation of the enriched KEGG pathway to determine the P value and standardized enrichment score.

Immunohistochemistry (IHC) and evaluation of immunostaining

The tissues were fixed in neutral buffered formalin (10%). After the tissues had been embedded tissues in paraffin, they were cut into sections. Antigen activity restoration was accomplished by boiling the sections in citrate buffer (10 mmol/L, pH 6.0) for 3 minutes in an autoclave after they had been deparaffinized in xylene and rehydrated with graded alcohol. A 3% hydrogen peroxide was employed to quench endogenous peroxidase activity. The antibodies employed for immunoassays included: anti-*EHD2* (1:100 dilution, ab222888, Abcam, Cambridge, MA, USA). Immunostaining was undertaken utilizing avidin-biotin-peroxidase complex method; meanwhile chromogenic reagent diaminobenzidine was used to observe the antigen-antibody reaction. The negative control slides were treated with phosphate-buffered saline (PBS). Without knowing the clinical and pathological parameters of LUAD patients, a blind method was used to evaluate all immunostained sections. To score *EHD2* staining, a semi-quantitative immune response scoring (IRS) system was implemented, which included distribution area and staining intensity. The immunostaining intensity score was 0 to 3 points (0, 1, 2, and 3 corresponded to no response, weak response, mild response, and strong response, respectively) and they were 1 (0–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). A final score could be attained by multiplying the intensity score by the proportional score. The results of 0–4 and 5–12 indicated low and high, respectively. The above evaluation process was carried out by 2 independent pathologists using a multi-head microscope, and the 2 pathologists collaborated to reach a consensus.

Western blot analysis

To extract proteins, we utilized radioimmunoprecipitation assay (RIPA) buffer (Beyotime, China, Shanghai) involving protease inhibitor cocktail as well as phosphatase inhibitor (Bimake, Houston, TX, USA), and to identify total protein concentrations, we employed Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). After polyacrylamide gel electrophoresis (PAGE), the proteins were transferred to polyvinylidene fluoride (PVDF) membrane before being incubated with a specific primary antibody overnight at 4 ℃. Anti-*EHD2* (ab222888), anti-Vimentin (ab92547), and anti-GAPDH (ab8245) were supplied by Abcam (Cambridge, MA, USA). Both anti-E-cadherin [14472] as well as anti-N-cadherin [13116] were provided by Cell Signaling Technology (Boston, MA, USA). Both goat anti-rabbit IgG-HRP (abs20040ss) as well as goat anti-mouse IgG-HRP (abs20039ss) were supplied by AiBiXin Biotechnology Co., Ltd. (Absin, China). The PVDF membrane was incubated with the secondary antibody for 1 hour at room temperature. The membrane was observed using ChemiDoc MP Imaging System from Bio-Rad, USA. We used ImageJ (National Institutes of Health, Bethesda, MD, USA) to analyze band intensity.

Cell culture and cell transfection

The cell lines A549, NCI-H1299, NCI-H1650, NCI-H1975, and NCI-H4006 were provided by Shanghai Institute of Cell Biology, and each cell line was authenticated. The above cell line was stored in Roswell Park Memorial Institute (RPMI)-1640 supplemented with 10% fetal bovine serum (FBS) and cultured at 37 ℃ in 5% CO_2. We used a lentiviral vector supplied by Shanghai Heyuan (Shanghai, China) to establish stable transfected cell lines. Then, we added 2 mg/mL puromycin to select cells. Finally, we used western blot to evaluate the transfection efficiency. The *EHD2* gene target sequences and short hairpin RNA (shRNA) sequences of *EHD2* are shown in *Table 1*.

Phagokinetic motility assays

After adding 1 mL of PBS (containing 20 μg of fibronectin) to each well of the 6-well plate, the wells were then subjected to 2 hours incubation at 37 ℃ in 5% CO_2. After washing with PBS, 86 μL of microspheres was added to 30 mL serum-free medium, gently pipetting to mix and homogenize, after which 2.5 mL of microglobulin-containing medium was added to each well. These were then incubated at 37 ℃ in 5% CO_2 for 1 hour, the cells were resuspended in a medium mixed with 0.05% FBS, and then 1,500 cells were poured into in each well and incubated for 18 hours. Finally, we observed the cell migration trajectory under the microscope and recorded the length of the movement.

Transwell migration and invasion assays

The cell migration and invasion tests were undertaken by

Table 1 Primer information used in quantitative real-time PCR

Gene	Sequences
EHD2	
Forward primer (5'-3')	5'-CGGAATTCCATGTTCAGCTGGCTG-3'
Reverse primer (5'-3')	5'-CGGGATCCCTCGGCGGAGCCCTT-3'
shRNA	
EHD2-shRNA1	5'-CTCCCTAATCAGGTCCTGGAGAG-3'
EHD2-shRNA2	5'-CTGCACGCACACCCCTGCTCCGG-3'
EHD2-shRNA3	5'-AAGAAAGAGATGCCCACGGTGTT-3'

PCR, polymerase chain reaction; *EHD2*, EH domain contains protein 2; shRNA, short hairpin RNA.

means of 24-well transwell plate (pore size 8 μm; Costar, Corning, NY, USA). Then, the upper chamber with a serum-free medium was charged with 5×10^4 cells, and the lower chamber was charged with a medium mixed with 10% FBS. Following 1-day incubation at 37 ℃ in 5% CO_2 incubator, the cells in upper chamber were stained with crystal violet. The average of cell counts observed in 5 random fields was utilized for calculating migrating cell number. Before adding the cells to the upper chamber, 50 μL of diluted Matrigel [Becton, Dickinson, and Co. (BD) Biosciences, Franklin Lakes, NJ, USA] was added.

Statistical analysis

All data were analyzed using the software SPSS 24.0 (IBM Corp., Armonk, NY, USA) and R 3.6.2 (The R Foundation for Statistical Computing, Vienna, Austria). To analyze the results of IHC staining and clinical parameters, the Pearson's chi-square test was employed. We defined OS as the time to death from any cause. The survival curve was determined using Kaplan-Meier approach, and the analysis was implemented utilizing log-rank test. To assess the associations between the *EHD2* expression and OS outcomes, hazard ratios (HRs) with 95% confidence intervals (CIs) were assessed by using univariate and multivariate Cox regression analyses. Adjustment variables included age, gender, smoking history, pathologic stage, T stage, and lymph node metastasis. Statistical significance was indicated by a 2-sided P value <0.05.

Results

Bioinformatics analysis of LUAD revealed that EHD2 could be a biomarker

The TIMER database was employed to analyze *EHD2* expression in different cancer types. The expression of *EHD2* was down-regulated in most cancers and reached the highest value in adjacent tissues of LUAD (*Figure 1*). We also observed in the GEPIA database that *EHD2* was significantly down-regulated in LUAD (*Figure 2A*), and it was significantly positively correlated with CDH1 expression (*Figure 2B*). Then, the Kaplan-Meier plotter indicated that down-regulating *EHD2* expression was linked to poor prognosis in LUAD patients (*Figure 2C*). The GSEA analysis demonstrated that *EHD2* may contribute to cell cycle progression, DNA replication, extracellular matrix (ECM) receptor interaction, focal adhesion, and homologous recombination (*Figure 2D*).

In addition, co-expression analysis was performed in TCGA-LUAD samples; co-expression was considered as $|cor|>0.4$, $P<0.05$ (*Figure 3A*). The GO and KEGG analyses revealed that *EHD2* was associated with focal adhesion, tight junction, cadherin binding, DNA replication, and adherens junction (*Figure 3B,3C*). These findings cumulatively implied that *EHD2* contributes to the EMT process of NSCLC and regulates radiosensitivity.

EHD2 expression and its link to clinicopathologic variables in LUAD cancer

To ascertain the role of *EHD2* in LUAD, after preparing 4 LUAD tissue samples, we used western blot analysis to detect *EHD2* expression. As illustrated in *Figure 4A*, LUAD tissues exhibited significantly lower *EHD2* expression than adjacent normal tissues. To further determine *EHD2* protein expression, we used IHC analysis to detect 170 tissue samples from LUAD patients. The *EHD2* immune response was mainly located on the cell membrane (*Figure 4B,4C*). The IHC findings showed that *EHD2* exhibited high expression in tissues without lymph node metastasis (*Figure 4B*). Nevertheless, tissues with lymph node metastasis had lower *EHD2* expression (*Figure 4C*). Low *EHD2* expression was intimately linked to advanced tumor-node-metastasis (TNM) stage (P=0.034) or lymph node metastasis (P=0.039) in LUAD (*Table 2*).

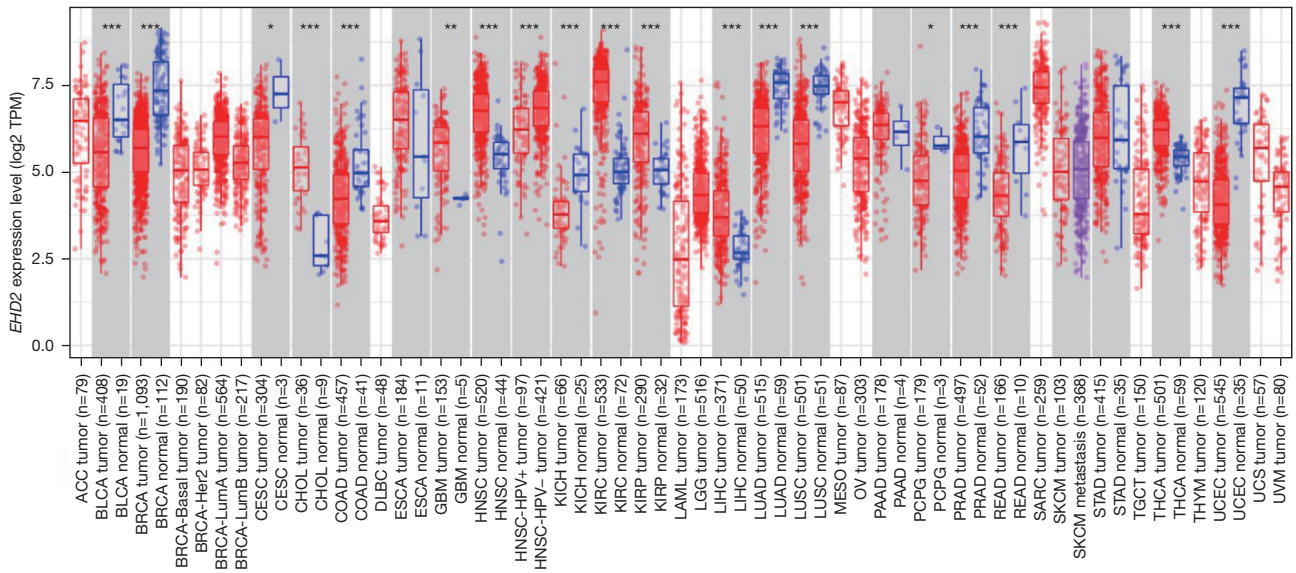

Figure 1 TIMER database analysis of the expression of *EHD2* in pan-cancer. *, P<0.05; **, P<0.01; ***, P<0.005. *EHD2*, EH domain contains protein 2; TIMER, tumor immune estimation resource. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; HPV, human papilloma virus; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, Mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Prognostic significance of EHD2 expression

For univariate analysis, the Kaplan-Meier survival curve revealed that, except for patients without adjuvant radiotherapy, low *EHD2* expression in other groups was significantly associated with low survival (*Figure 5A-5C*). The Cox proportional hazards regression model confirmed that independent prognostic factors for LUAD patients included *EHD2* expression and TNM stage (*Table 3*). The nomogram which was established on the basis of the Fine and Gray models is displayed in *Figure 6A*. The calibration chart showed that these points were very close to the blue line, indicating a high degree of agreement between the predicted and actual survival events (*Figure 6B,6C*).

EHD2 suppresses EMT in LUAD cells

The expression of *EHD2* in LUAD cell lines (A549, H1299, H1650, H1975, and H4006) was explored, demonstrating higher expression in A549 cells than in H1299, H1650, H1975, and H4006 cells (*Figure 7A*). Then, we used lentivirus-mediated infection to knockdown and overexpress *EHD2* in the cell lines H1299 and A549. We used shRNA3 (*EHD2* shRNA) in subsequent experiments because it was the most effective at knocking down *EHD2* (*Figure 7B*). This comprehensive analysis indicated a link between *EHD2* expression and lymph node metastasis. Therefore, we inferred that *EHD2* contributes to migration and invasion inhibition. Transwell assays and phagocytic motility assays revealed that *EHD2* knockdown markedly contributed to an increase in migration and invasion in A549 cells. By contrast, *EHD2* overexpression substantially suppressed H1299 cell migration and invasion (*Figure 7C,7D*). Western blot analysis was executed to identify EMT markers, including E-cadherin, N-cadherin, and vimentin. The results indicated decreased E-cadherin

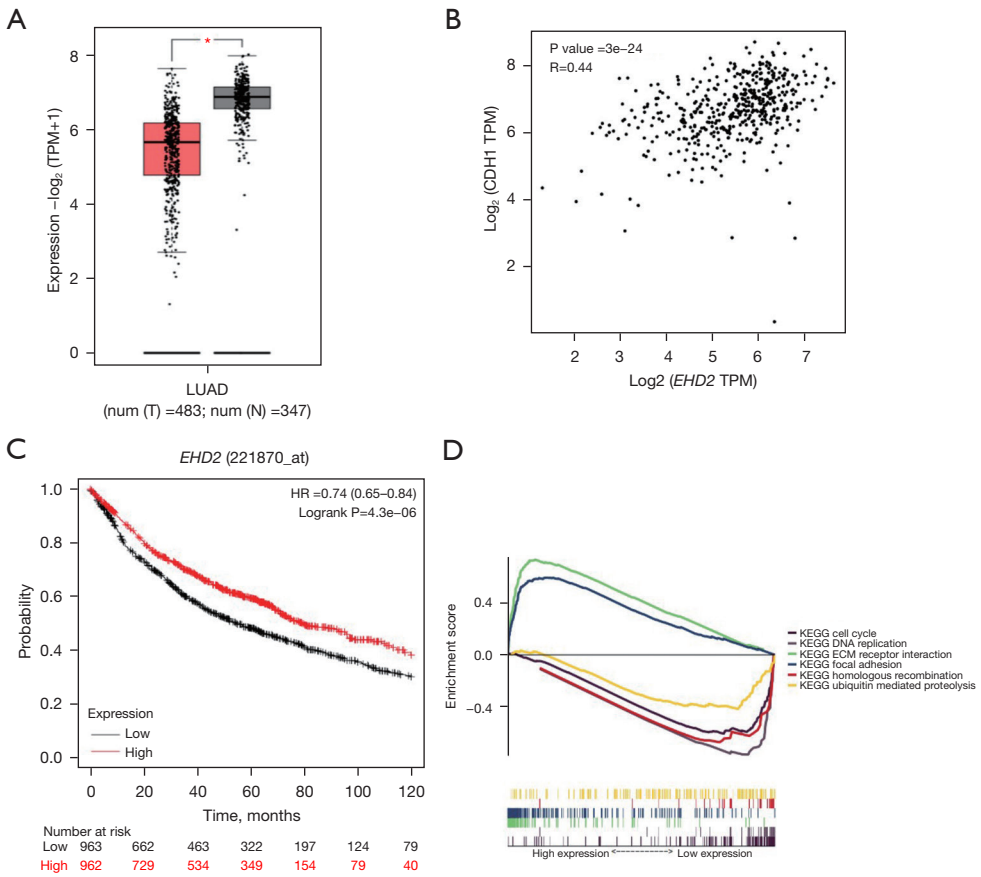

Figure 2 Integrated analysis of LUAD reveals that *EHD2* may be a biomarker. (A) GEPIA analyzes of the expression of *EHD2* in LUAD. Red represents tumor tissue group, and black represents adjacent normal tissue group; (B) GEPIA analyzes the correlation between *EHD2* and CDH1; (C) Kaplan-Meier plotter analysis of the relationship between *EHD2* and the prognosis of LUAD patients; (D) GSEA-KEGG pathway analysis. *, P<0.05. TPM, transcripts per million; LUAD, lung adenocarcinoma; T, tumor; N, normal; *EHD2*, EH domain contains protein 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; ECM, extracellular matrix; GEPIA, Gene Expression Profiling Interactive Analysis; GSEA-KEGG, gene set enrichment analysis-Kyoto Encyclopedia of Genes and Genomes.

expression in A549 cells with *EHD2* knockdown, as well as increased N-cadherin and vimentin expressions. By contrast, E-cadherin expression was increased in H1299 cells, whereas N-cadherin and vimentin expressions were decreased in response to *EHD2* overexpression (*Figure 7E*). These findings demonstrated that *EHD2* suppressed EMT of LUAD.

Discussion

Lung cancer is one of the world's most dangerous malignant tumors, posing a threat to human health and life. In terms of lung cancer cases, NSCLC is the most prevalent, accounting for up to 90% of all cases, and LUAD has become the prevalent type of NSCLC. Radiotherapy is one of the traditional clinical treatment options for NSCLC, but due to poor targeting and side effects, the prognosis of patients has been poor (18). Therefore, finding novel therapeutic targets has important therapeutic significance.

EHD2 is a member of EHD protein family with the lowest conservation degree, which is responsible for the membrane trafficking between the endosomes and plasma membrane (19,20). In muscle cells, *EHD2* has been shown to be involved in membrane resealing/fusion, which can regulate actin function in several cell structures (20). These associations may change in normal cells adjacent to the tumor or in malignant cells, thereby promoting invasion,

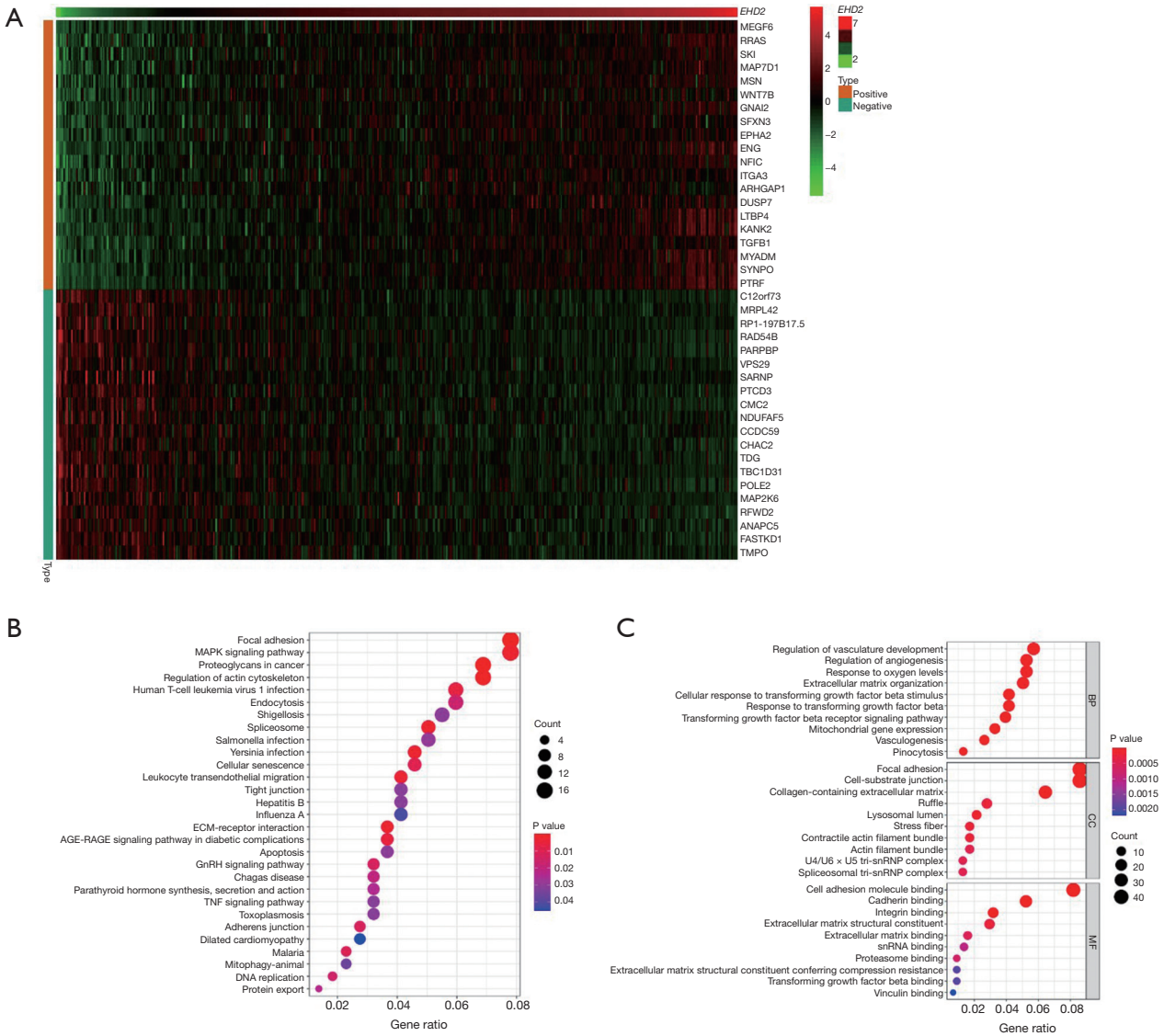

Figure 3 Co-expression analysis of *EHD2*. (A) The top 20 genes whose co-expression was positive and negative with *EHD2*; (B) KEGG enrichment analysis; (C) GO analysis. *EHD2*, EH domain contains protein 2; BP, biological processes; CC, cellular components; MF, molecular functions; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

metastasis, and colonization (21). Previous studies have shown that *EHD2* may serve as a prognostic marker in breast cancer, papillary thyroid carcinoma, esophageal squamous cell carcinoma, and CRC (15,17,21,22). Furthermore, Shen *et al.* reported that low *EHD2* levels were associated with enhanced proliferation, migration, and invasion of triple negative breast cancer (TNBC) cells, and good prognosis in the highly proliferative TNBC subtype (23). In line with previous studies, we found that *EHD2* was down regulated in LUAD tissues and correlated with poor prognosis through comprehensive bioinformatics analysis. Notably, while *EHD2* expression was high in tissues free of lymph node metastasis in the present study, it was low in lymph node metastasis tissues. The decreased expression of *EHD2* was associated with advanced TNM stage or lymph node metastasis. Furthermore, the multivariate Cox analysis showed that *EHD2* expression levels were independent risk factors for survival following radical resection. These findings provide, for the first time, evidence that *EHD2* may be a useful prognostic biomarker for LUAD.

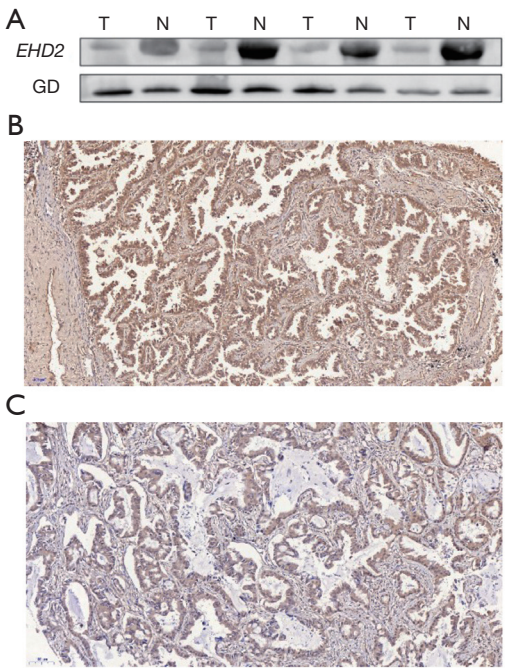

Figure 4 Expression of *EHD2* in LUAD tissues (A) Western blot analysis of *EHD2* expression in lung adenocarcinoma; (B) IHC staining of *EHD2* in patients without lymph node metastasis; (C) IHC staining of *EHD2* in patients with lymph node metastasis. All immunostained sections were imaged at 100× magnification. T, tumor; N, normal; *EHD2*, EH domain contains protein 2; GD, GAPDH; IHC, immunohistochemistry.

Table 2 Clinicopathology characteristics of patients

Variables	Patients	*EHD2* low, n (%)	*EHD2* high, n (%)	P value
Gender				0.518
Female	69	30 (43.5)	39 (56.5)	
Male	101	49 (48.5)	52 (51.5)	
Age (years)				0.398
<65	34	18 (52.9)	16 (47.1)	
≥65	136	61 (44.9)	75 (55.1)	
Smoking history				0.780
No	135	62 (45.9)	73 (54.1)	
Yes	35	17 (48.6)	18 (51.4)	
Pathologic stage				0.034*
I–II	117	48 (41.0)	69 (59.0)	
III–IV	53	31 (58.5)	22 (41.5)	
T stage				0.868
T1 and T2	145	67 (48.9)	78 (51.1)	
T3 and T4	25	12 (72.7)	13 (27.3)	
Lymph node metastasis				0.039*
No	94	37 (39.4)	57 (60.6)	
Yes	76	42 (55.3)	34 (44.7)	

*, P<0.05. *EHD2*, EH domain contains protein 2.

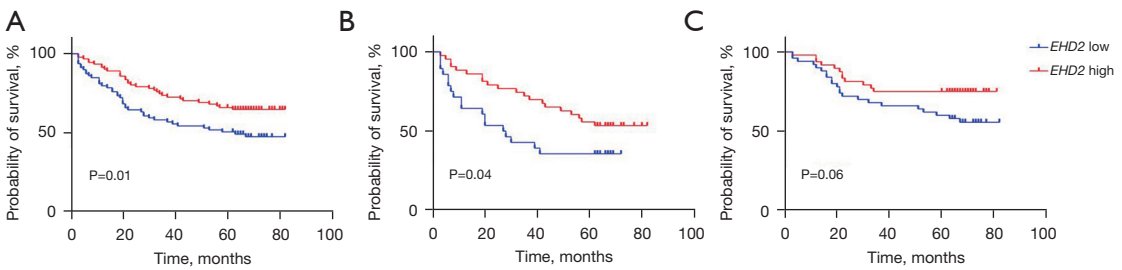

Figure 5 Association of *EHD2* expression with the overall survival of HCC patients by Kaplan-Meier analysis. (A) All sample; (B) patients who have received radiotherapy; (C) patients who have not received radiotherapy. *EHD2*, EH domain contains protein 2; HCC, hepatocellular carcinoma.

EHD2 is implicated in regulating cell membrane transport, and is related to signal transduction regulation, as well as actin cytoskeleton and transcriptional regulation of endocytic pathway (11,24,25). The current research sought to explore the role of *EHD2* in LUAD and its underlying molecular mechanism. The GSEA analysis showed that *EHD2* may contribute to cell cycle progression, DNA replication, ECM receptor interaction, focal adhesion, and homologous recombination. *In vitro*, the present results showed that *EHD2* knockdown markedly contributed to an increase in migration and invasion in LUAD cells and *EHD2* overexpression substantially suppressed migration and invasion in LUAD cells. However, the present study did not assess the effects of *EHD2* on proliferation or

Table 3 Univariate and multivariate analysis of OS

Variables	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender	1.176 (0.733–1.884)	0.502	–	–
Age (years)	1.080 (0.603–1.934)	0.796	–	–
Smoking history	1.151 (0.661–2.004)	0.619	–	–
Pathologic stage	3.367 (2.120–5.346)	<0.01*	3.284 (2.065–5.223)	<0.01*
T stage	2.009 (1.137–3.551)	0.016*	–	–
Lymph node metastasis	2.092 (1.311–3.337)	<0.01*	–	–
EHD2 expression	0.572 (0.360–0.908)	0.018*	0.605 (0.381–0.963)	0.034*

*, P<0.05. OS, overall survival; HR, hazard ratio; CI, confidence interval; *EHD2*, EH domain contains protein 2.

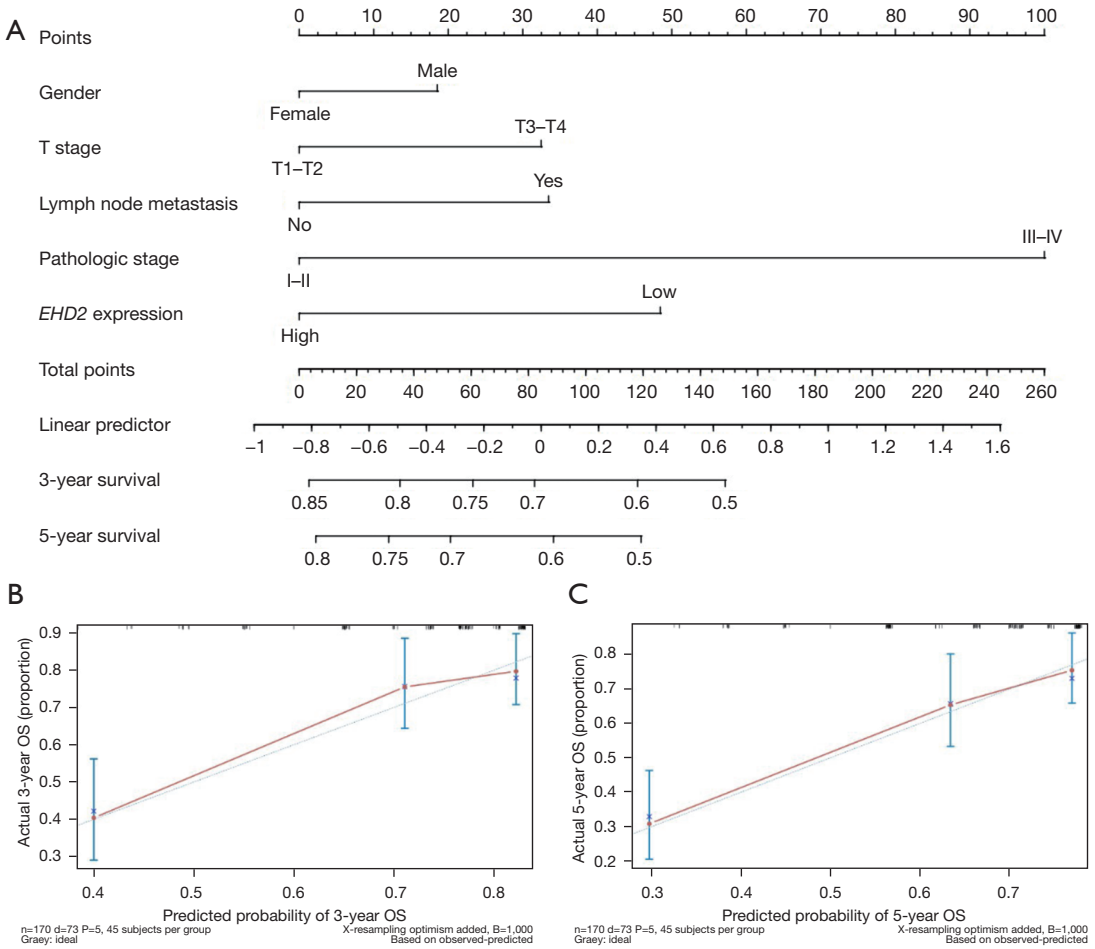

Figure 6 The constructed nomogram for prognostic prediction of patients with LUAD. (A) A nomogram for predicting the 3- and 5-year survival probability of patients with LUAD; (B) calibration curve showing nomogram performance (3-year); (C) calibration curve showing nomogram performance (5-year). *EHD2*, EH domain contains protein 2; OS, overall survival; LUAD, lung adenocarcinoma.

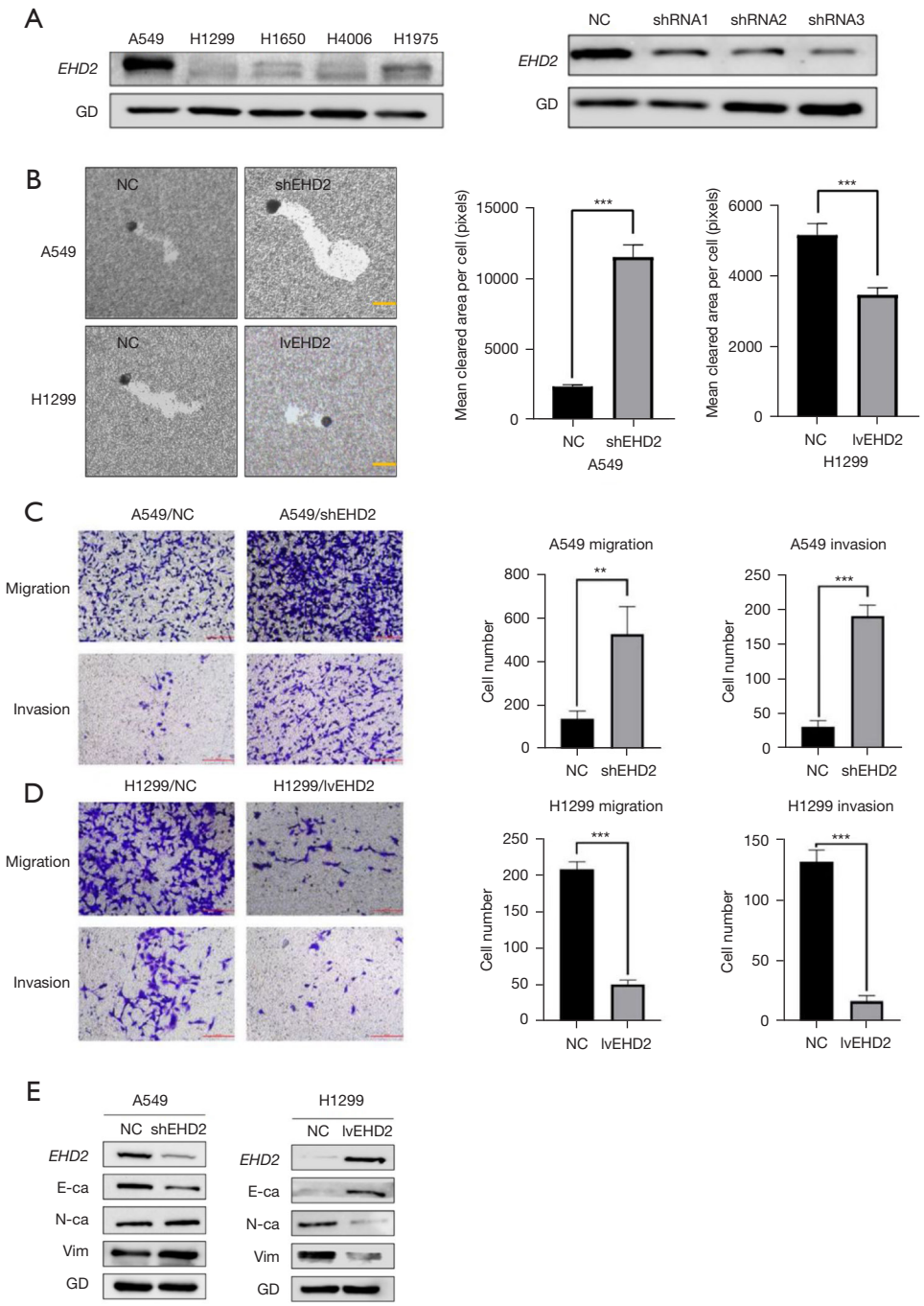

Figure 7 *EHD2* expression affects lung adenocarcinoma migration and invasion ability. (A) The expression of *EHD2* in LUAD cells and shRNA-mediated *EHD2* knockdown cells; (B) Phagokinetic motility assays in H1299 and A549 cells (100×); (C) Transwell in A549 cells; (D) Transwell in H1299 cells; (E) the expression of *EHD2*, E-cadherin, N-cadherin, and Vimentin were explored by western blot. All crystal violet staining sections were imaged at 100× magnification (C,D). **, P<0.01 *vs.* control; ***, P<0.005 *vs.* control. *EHD2*, EH domain contains protein 2; GD, GAPDH; NC, normal control; E-ca, E-cadherin; N-ca, N-cadherin; Vim, Vimentin; LUAD, lung adenocarcinoma.

apoptosis in LUAD. By contrast, Liu *et al.* demonstrated that *EHD2* overexpression enhanced the proliferation, invasion, and migration but inhibited the apoptosis of clear cell renal cell carcinoma (ccRCC) cells, while *EHD2* interference showed opposite functions. Interference of *EHD2* can inhibit the development of ccRCC by inhibiting the cellular proliferation, invasion, and migration (26). In addition, *EHD2* overexpression inhibits colon cancer cell proliferation, but enhances cell apoptosis and cell cycle arrest (15). Regarding the role of *EHD2* on proliferation or apoptosis in LUAD needs to be addressed in future studies.

In the present study, we also used western blot analysis to identify EMT markers, and the results showed that *EHD2* increased E-cadherin expression, whereas it reduced the expressions of N-cadherin and vimentin. Previous studies have indicated that *EHD2* knockdown promotes EMT, while *EHD2* overexpression inhibits EMT in osteosarcoma and liver cancer (13,27). Several studies have demonstrated that EMT promotes early epithelial cancer cell diffusion and is an essential parameter in epithelial cancer invasion and metastasis (28,29). In addition, cancer cells undergoing EMT tend to be resistant to various anti-cancer treatments (30-32). To our knowledge, *EHD2* regulates actin recombination to promote endocytosis by controlling Rac1 activity in tumorigenesis. *Rac1* regulates the maintenance of cell polarity and cell migration by regulating GTPase activity and cytoskeleton rearrangement. It is an important signal transduction and polarity regulator in cells (10,15). This regulation of activity may significantly affect EMT, which is closely related to tumor cell invasion and distant metastasis (33). Furthermore, E-cadherin, as a calcium dependent cell adhesion molecule, can mediate the adhesion of allogeneic cells (34). The abnormal expression of E-cadherin is related to tumor differentiation, metastasis, invasion, and prognosis (35). With the decrease or disorder of E-cadherin expression, the ability of tumor invasion and metastasis will be enhanced (36,37). Given the above findings, *EHD2* may have the potential to serve as therapeutic target for LUAD.

In conclusion, our study demonstrated that *EHD2* reduces LUAD cell migration and invasion by preventing EMT. The expression level of *EHD2* can be used as an independent prognostic factor for the survival of LUAD.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-842/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-842/coif). The authors have no conflicts of interest to declare.

Ethics 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). This study was approved by the Ethics Committee of the Affiliated Tumor Hospital of Nantong University (No. 2016-094). All participants provided written informed consent.

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