Received: 26 August 2018 | Accepted: 25 September 2018

DOI: 10.1002/jcb.27915

RESEARCH ARTICLE

WILEY Journal of Cellular Biochemistry

The clinical and prognostic significance of YWHAZ in non-small–cell lung cancer patients: Immunohistochemical analysis

Yong Deng[1] | **Jianyun Zheng**[2] | **Jiangang Ma**[3]

[1]Department of Thoracic Surgery, Sheyang County People's Hospital, Yancheng, China

[2]Department of Pathology, The First Affiliated Hospital of Xi'an Medical University, General Medicine School of Xi'an Medical University, Xi'an, China

[3]Department of Respiratory Medicine, The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, China

Correspondence
Jiangang Ma, Department of Respiratory Medicine, The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, No. 5 Weiyang Road West, Xianyang 712000, Shaanxi, China.
Email: majiangangxy@126.com

Abstract

YWHAZ has been suggested to as an oncogene in various human malignancies, including non-small–cell lung cancer (NSCLC). Our study presents more evidence to confirm the clinical significance and biological function of YWHAZ in NSCLC. In our results, YWHAZ was upregulated in lung squamous cell carcinoma tissues and lung adenocarcinoma tissues through analyzing The Cancer Genome Atlas (TCGA) database, and confirmed high levels of YWHAZ messenger RNA and protein in lung squamous cell carcinoma tissues and lung adenocarcinoma tissues through quantitative real-time polymerase chain reaction and immunohistochemistry. Moreover, YWHAZ overexpression was correlated with advanced clinical stage, more lymph node metastasis and present distant metastasis in NSCLC patients. Survival analysis indicated that high level of YWHAZ protein expression was associated with short overall survival time in NSCLC patients, and YWHAZ expression was independent prognostic factors for overall survival in NSCLC patients. Moreover, Silencing of YWHAZ expression represses NSCLC cell migration and invasion. In conclusion, YWHAZ is a credible prognostic biomarker, and may be a therapeutic target in NSCLC.

KEYWORDS

14-3-3, biomarker, lung cancer, prognosis, YWHAZ

1 | INTRODUCTION

Lung cancer is a most common human malignancy and the leading cause of cancer-related mortality accounting for about 27.3% of all cancer deaths per year in China.[1] Recent cancer statistics published at 2018 by National Central Cancer Registry of China showed about 782 000 newly diagnosed lung cancer patients and 626 000 lung cancer deaths were appeared in China.[1] Non-small–lung cancer (NSCLC) including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), lung large-cell carcinoma, and lung adenosquamous carcinoma are the major subtype of lung cancer accounting for approximately 80% of all lung cancer cases.[2] Despite recent advances in prevention, screening, and treatment, the prognosis is still unfavorable (5-year overall survival rate <15% and 10-year overall survival rate <7%).[3] Therefore, it is necessary to screen and identify novel functional genes for illuminating the pathogenesis and developing target therapy in NSCLC.

The YWHAZ gene is located on chromosome 8q22.3 and this area is frequently amplified in human cancers.[4] YWHAZ has been found to be overexpressed and functions as an oncogene in most human malignancies.[5] Five years ago, the clinical and prognostic value of YWHAZ was still known. Initially, we analyzed The Cancer Genome Atlas (TCGA) database, and found

Yong Deng and Jianyun Zheng are co-first authors.

YWHAZ was upregulated in NSCLC tissues, and associated with overall survival in NSCLC patients. Therefore, we supposed YWHAZ acts as an oncogene in NSCLC, and began to study the clinical significance of YWHAZ in Chinese NSCLC patients and biological function of YWHAZ in NSCLC cells. Recent studies reported YWHAZ was overexpressed in LUAD and LUSC tissues, and associated with the malignant status and poor prognosis in LUAD and LUSC patients. In our study, we present more evidence indicating that levels of YWHAZ messenger RNA (mRNA) and protein are increased in NSCLC clinical tissue samples, and high-expression of YWHAZ was correlated with clinical progression and unfavorable prognosis in NSCLC patients. Moreover, downregulation of YWHAZ expression inhibited NSCLC cell migration and invasion.

2 | MATERIALS AND METHODS

2.1 | Analysis of TCGA database

Gene expression data of TCGA projects were analyzed and downloaded from a visualization website Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/). GEPIA contained differential gene expression between human cancer tissues and corresponding normal tissues, and the relationship between gene expression and overall survival time.

2.2 | Clinical tissue specimens

All protocols were approved by the Research Ethics Committee of Sheyang County People's Hospital, The First Affiliated Hospital of Xi'an Medical University and The Second Affiliated Hospital of Shaanxi University of Chinese Medicine. All of the clinical tissues were derived from patients having given informed consent in accordance with Ethics Committee Guidelines and the Declaration of Helsinki. A total of 152 NSCLC tissue samples and 30 noncancerous lung tissue samples were collected from Sheyang County People's Hospital, The First Affiliated Hospital of Xi'an Medical University and The Second Affiliated Hospital of Shaanxi University of Chinese Medicine. All clinical tissue specimens were diagnosed as NSCLC by pathologists. No patient had received chemotherapy or radiotherapy before pathologic diagnosis.

2.3 | RNA preparation and quantitative real-time polymerase chain reaction

Total RNA from tissues or cells was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The total RNA was reverse-transcribed to complementary DNA using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (RT-qPCR) was performed at ABI 7500 system (Applied Systems, Foster, CA) using the SYBR Premix Ex Taq II (Takara) with the follow primers. YWHAZ, forward primer: 5′-ACTTTTG GTACATTGTGGCTTCAA-3′ and reverse primer: 5′-CCGCCAGGACAAACCAGTAT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward primer: 5′-CAGCCTCAAGATCATCAGCA-3′ and reverse primer: 5′-TGTGGTCATGAGTCCTTCCA-3′. GAPDH was used as an internal control.

2.4 | Immunohistochemistry

Formalin-fixed paraffin-embedded sections (4-μm-thick) were deparaffinized through graded alcohols subjected to microwave pretreatment for antigen retrieval. After cooling to room temperature, each section was blocked for endogenous peroxidase activity by incubation with 0.3% H_2O_2 for 10 minutes. Nonspecific binding sites were blocked by incubation with 2% fetal bovine serum (FBS) for 15 minutes. Next, sections were incubated with anti-YWHAZ (1:100 dilution; Abcam, Cambridge, UK) overnight at 4°C. The primary antibody was replaced with phosphate-buffered saline in sections used as negative controls. After washing, incubation with biotinylated antimouse immunoglobulin G as the secondary antibody was carried out for 30 minutes, followed by incubation with conjugated horseradish peroxidase streptavidin for 30 minutes. Finally, sections were then counterstained with hematoxylin, dehydrated, and mounted. For YWHAZ assessment, staining intensity was scored as 0: negative; 1: weak; 2: moderate; or 3: strong, and staining extent was scored as 0: 0%; 1: 1% to 23%; 2: 25% to 49%; 3: 50% to 74%; or 4: 75% to 100%.[6] The final scores were defined by multiplying the intensity scores with the scores of the extent of stained cells. All cases was classified into low-expression of YWHAZ group (0 to 4 scores) and high-expression of YWHAZ group (>4 scores).

2.5 | Cell culture and transfection

Two human NSCLC cell lines A549 (lung adenocarcinoma cell line) and SK-MES-1 (lung squamous cell carcinoma cell line) were obtained from Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The A549 and SK-MES-1 cells were maintained in Dulbecco modified Eagle medium (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% FBS in a humidified air atmosphere with 5% CO_2 at 37°C.

The small interfering RNA targeting YWHAZ (si-YWHAZ) was synthesized by GenePharma Co, Ltd, (Shanghai, China) for downregulation of YWHAZ, with si-NC as a negative control. NSCLC cells were transfected with si-YWHAZ or si-NC using Lipofectamine 3000 (Invitrogen) according to manufacturer's protocol.

2.6 | Invasion and migration assays

The transwell invasion and migration assays were conducted using the 24-well transwell chambers (8-μm-pore size; BD Biosciences, Franklin Lakes, NJ). For migration assay, transfected cells (5×10^4 cells in 0.2 mL RPMI-1640 medium per well) were added to the upper chambers, while 20% FBS in 0.5 mL RPMI-1640 medium was placed into the lower chambers as the chemoattractant. After 24 hours of incubation, cells remaining on the upper membrane were removed carefully by cotton swab, and adherent to underside of the membrane were fixed and stained with crystal violet. The number of migratory cells was counted from five random fields under a microscope. For the invasion assay, the upper chambers were coated with thin layers of Matrigel (BD Biosciences), and the experiment was similar to the migration assay described above.

2.7 | Statistical analysis

The SPSS 18.0 (Chicago, IL) and Graphpad Prism (San Diego) softwares were used for statistical analyses. Data were expressed as mean ± SD. Association between the clinicopathological parameters and YWHAZ expression was assessed by χ^2 test. The statistical significance between two groups was estimated by two-sided Student t test. Overall survival curves were calculated using the Kaplan-Meier method with significance evaluated by two-sided log-rank test. The independent prognostic relevance of YWHAZ was evaluated by univariate and multivariable Cox regression models. A $P < 0.05$ was regarded as the statistical significance.

3 | RESULTS

3.1 | YWHAZ is overexpressed in NSCLC

To evaluate the expression status of YWHAZ in NSCLC, we observed YWHAZ expression in TCGA database which included 483 LUAD tissue samples, 486 LUSC tissue samples and 347 normal lung samples. Compared with normal lung tissue samples, significantly elevated YWHAZ expression was shown in LUAD tissue samples or LUSC tissue samples (both $P < 0.001$; Figure 1A). Furthermore, qRT-PCR was used to detect YWHAZ mRNA transcript levels in LUAD tissues, LUSC tissues, and normal lung tissues. LUAD tissues and LUSC tissues exhibited an obviously higher-level expression of YWHAZ mRNA compared with normal lung tissues (both $P < 0.001$; Figure 1B). Moreover, YWHAZ protein was examined by immunohistochemistry in 152 NSCLC tissue samples and 30 noncancerous lung tissue samples. Specific YWHAZ protein staining was detected in the nuclei and cytoplasm of noncancerous and malignant epithelial cells (Figure 2A-I). Compared with noncancerous lung tissue samples, increased expression of YWHAZ protein was observed in LUAD tissue samples ($P = 0.001$; Table 1) and LUSC tissue samples ($P = 0.019$; Table 1).

3.2 | Immunohistochemical analysis of the clinical significance of YWHAZ expression in NSCLC patients

To evaluate the clinical significance of YWHAZ in NSCLC cases, the associations between clinicopathological

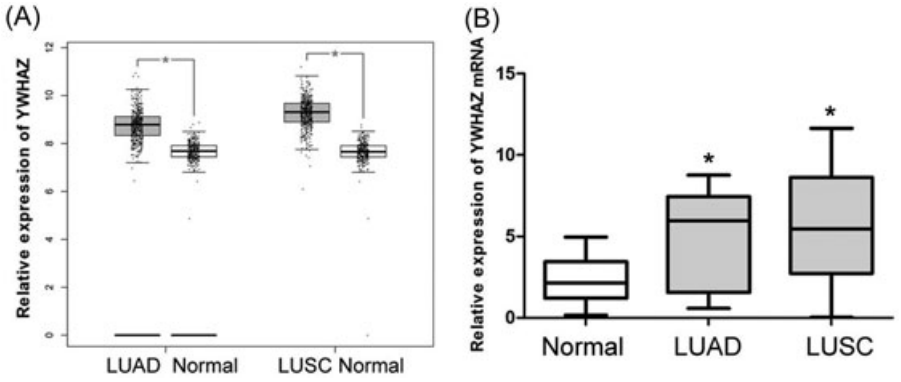

FIGURE 1 YWHAZ expression is increased in NSCLC tissues. A, YWHAZ expression is significantly elevated in LUAD tissue samples and LUSC tissue samples compared with normal lung tissue samples from TCGA database. B, LUAD tissues and LUSC tissues exhibited an obviously higher-level expression of YWHAZ mRNA compared to normal lung tissues (*$P < 0.001$). LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; mRNA, messenger RNA; NSCLC, non-small–cell lung cancer; TCGA. The Cancer Genome Atlas

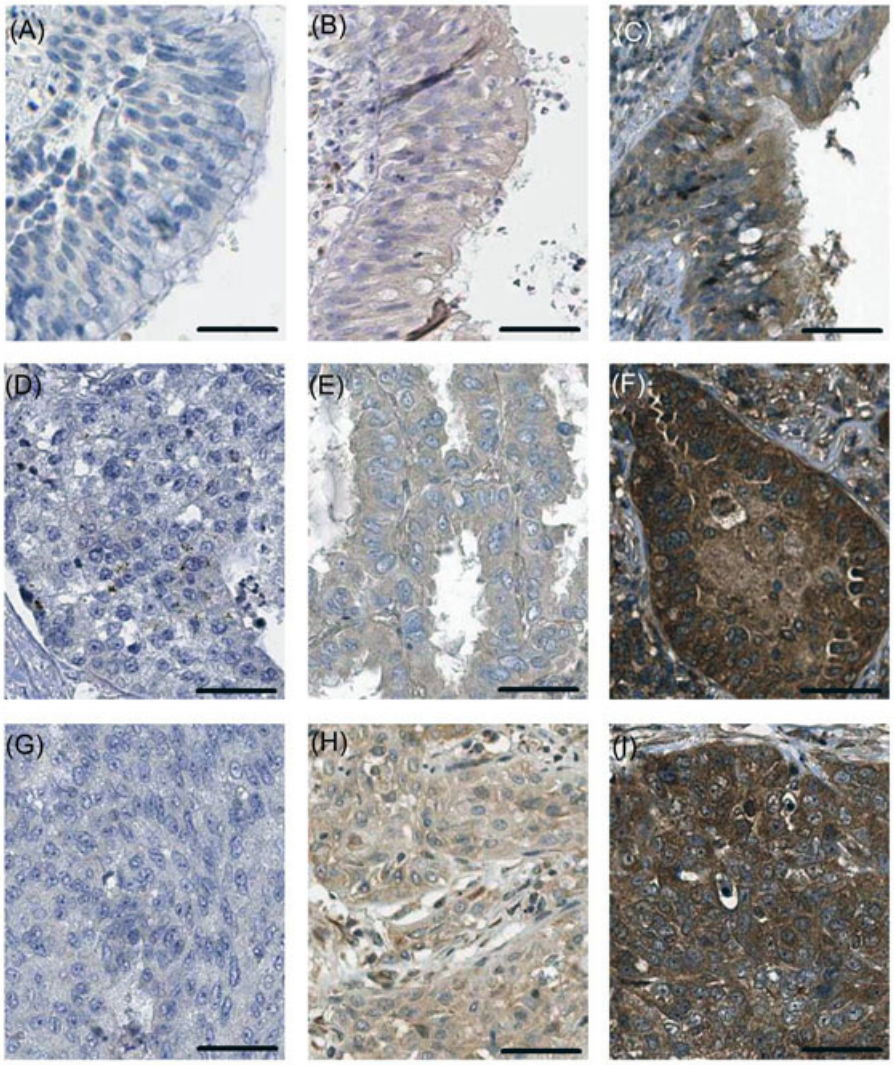

FIGURE 2 Immunohistochemical staining of YWHAZ. A, Negative expression, (B) low expression, (C) high expression of YWHAZ in normal bronchial epithelium tissues, (D) negative expression, (E) low expression, (F) high expression of YWHAZ in LUAD tissues, (G) negative expression, (H) low expression, and (I) high expression of YWHAZ in LUSC tissues. Scalebars = 50 μm. LUSC, lung squamous cell carcinoma

characteristics and YWHAZ protein expression were analyzed and summarized in Table 2. We observed that YWHAZ overexpression was correlated with advanced clinical stage ($P < 0.001$), more lymph node metastasis ($P < 0.001$), and present distant metastasis ($P = 0.001$). However, we did not observed a significant relationship between YWHAZ expression with NSCLC patient's sex ($P = 0.165$), age ($P = 0.557$), smoking ($P = 0.318$), pathology classification ($P = 0.176$), and tumor size ($P = 0.181$).

TABLE 1 YWHAZ protein expression in lung cancer tissues and normal lung tissues

		YWHAZ protein		
Group	Cases	High expression	Low expression	*P*
Normal tissues	30	7	23	–
Adenocarcinoma tissues	84	50	34	0.001[a]
Squamous cell carcinoma tissues	68	33	35	0.019[a]

[a]compared with normal tissues.

3.3 | Survival analysis of YWHAZ expression in NSCLC patients

To evaluate the prognostic significance of YWHAZ in NSCLC cases, the associations between overall survival time and YWHAZ expression were evaluated in TCGA database and our study. In TCGA database, we observed that the high level of YWHAZ protein expression was markedly associated with short overall survival time in NSCLC patients ($P = 0.001$; Figure 3A). In a subgroup analysis of TCGA database, we found high-expression of YWHAZ protein was correlated with overall survival in LUAD cases ($P < 0.001$; Figure 3B), but had no correlation with overall survival in LUSC cases ($P = 0.550$; Figure 3C). In our study, we found YWHAZ protein levels were negatively correlated with overall survival time in NSCLC patients ($P < 0.001$; Figure 3D). In a subgroup analysis of our study, we

TABLE 2 Asocciation between the clinicopathologic variables and expression of YWHAZ in NSCLC patients

Characteristics	*n*	YWHAZ protein		*P*
		High expression	Low expression	
Sex				
Female	57	27	30	0.165
Male	95	56	39	
Age, y				
< 50	60	31	29	0.557
≥50	92	52	40	
Smoking				
No	86	50	36	0.318
Yes	66	33	33	
Pathology classification				
Squamous cell carcinoma	68	33	35	0.176
Adenocarcinoma	84	50	34	
Clinical stage				
I-II	65	18	47	<0.001
III-IV	87	65	22	
Tumor size, cm				
≤5	88	44	44	0.181
>5	64	39	25	
Lymph node metastasis				
N0-N1	72	23	49	<0.001
N2-N3	80	60	20	
Distant metastasis				
No	139	70	69	0.001
Yes	13	13	0	

Abbreviation: NSCLC, non-small–cell lung cancer.

observed high levels of YWHAZ protein expression was significantly associated with short overall survival time in LUAD patients ($P < 0.001$; Figure 3E) and in LUSC patients ($P = 0.016$; Figure 3F). Furthermore, the univariate Cox regression analysis suggested that clinical stage ($P < 0.001$), tumor size ($P = 0.008$), lymph node metastasis ($P < 0.001$), distant metastasis ($P < 0.001$), and YWHAZ protein expression ($P < 0.001$) were poor prognostic factors for overall survival in NSCLC patients (Table 3). Clinical parameters that exhibited significant differences in the univariate analysis were included in multivariate analysis. Then, the results of multivariate analysis showed YWHAZ expression ($P = 0.012$) and distant metastasis ($P = 0.002$) were independent prognostic factors for overall survival in NSCLC patients (Table 3).

3.4 | YWHAZ negatively regulates NSCLC cell migration and invasion

To estimate the effect of YWHAZ on NSCLC cell migration and invasion in vitro, the loss-of-function study was conducted in A549 and SK-MES-1 cells through si-YWHAZ (Figure 4A). To explore the role of YWHAZ in cell motility and migration, the transwell migration and invasion assays were conducted in A549 and SK-MES-1 cells. We observed that silencing of YWHAZ expression repressed cell migration in A549 and SK-MES-1 cells ($P < 0.001$; Figure 4B), and the invasive ability of YWHAZ-silenced cells was also reduced ($P < 0.001$; Figure 4C).

4 | DISCUSSION

YWHAZ is a member of the family of 14-3-3 proteins, and located on chromosome 8q22.3.[5] Recent decade, YWHAZ has been found upregulated in gastric cancer,[7] breast cancer,[8] colorectal cancer,[9] hepatocellular carcinoma,[10] pancreatic cancer,[11] prostate cancer,[12] bladder cancer,[13] ovarian cancer,[14] cervical cancer,[15] oral squamous cell carcinoma,[16] laryngeal cancer,[17] and chronic myeloid leukemia.[18] In lung cancer, Zhan et al[19] report YWHAZ exhibited high expression level in lung squamous cell carcinoma samples compared with normal lung samples. Moreover, Tong et al[20] also showed YWHAZ expression was increased in lung adenocarcinoma tissues compared with and corresponding adjacent normal tissues. In our study, we found YWHAZ was upregulated in lung squamous cell carcinoma tissues and lung adenocarcinoma tissues through analyzing TCGA database, and confirmed high levels of YWHAZ mRNA and protein in lung squamous cell carcinoma tissues and lung adenocarcinoma tissues through qRT-PCR and immunohistochemistry. Interestingly, Chen et al[21] suggested urinary protein of YWHAZ expression was decreased in NSCLC cancer patients. Generally, YWHAZ is overexpressed in most human cancer tissues.

In our study, we further evaluate the clinical significance of YWHAZ in NSCLC cases through analyzing the associations between clinicopathological characteristics and YWHAZ protein expression, and found YWHAZ overexpression was correlated with advanced clinical stage, more lymph node metastasis and present distant metastasis. Similarly, Tong et al[20] and Zhao et al[22] also found high-expression of YWHAZ was associated with clinical progression in lung squamous cell carcinoma and lung adenocarcinoma patients. Moreover, Nishimura et al[23] indicated overexpression of YWHAZ was related with deep tumor depth, large tumor size,

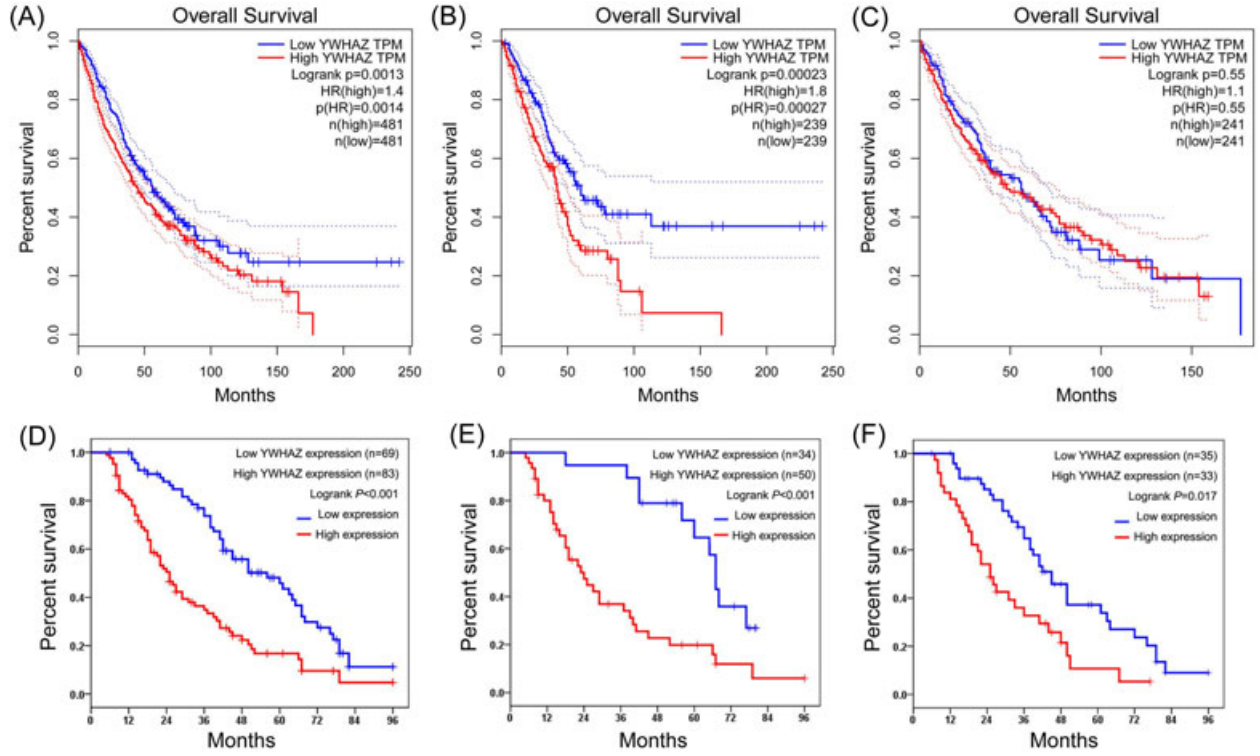

FIGURE 3 Survival analysis of YWHAZ expression in NSCLC patients. The association between overall survival time and YWHAZ expression was evaluated in NSCLC patients (A), LUAD (B) and LUSC (C) from TCGA database. The relationship between overall survival time and YWHAZ protein expression was estimated in NSCLC patients (D), LUAD (E) and LUSC (F) from our study. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small–cell lung cancer; TCGA, The Cancer Genome Atlas

TABLE 3 Summary of univariate and multivariate Cox regression analyses of overall survival duration in NSCLC patients

	Univariate analysis			Multivariate analysis		
Parameters	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**
Sex Female vs male	0.497	1.147	0.772-1.702	–	–	–
Age, y <50 vs ≥50	0.411	0.853	0.585-1.246	–	–	–
Smoking No vs yes	0.732	1.069	0.731-1.562	–	–	–
Pathology classification Squamous cell carcinoma vs adenocarcinoma	0.182	1.296	0.886-1.898	–	–	–
Clinical stage I-II vs III-IV	<0.001	2.813	1.848-4.284	0.360	0.502	0.115-2.193
Tumor size, cm ≤5 vs > 5	0.008	1.724	1.171-2.537	0.066	1.492	0.973-2.287
Lymph node metastasis N0-N1 vs N2-N3	<0.001	3.094	2.044-4.682	0.055	4.088	0.969-17.245
Distant metastasis No vs yes	<0.001	6.658	3.440-12.888	0.002	3.066	1.507-6.238
YWHAZ expression High vs low	<0.001	2.374	1.614-3.392	0.012	1.748	1.132-2.699

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio.

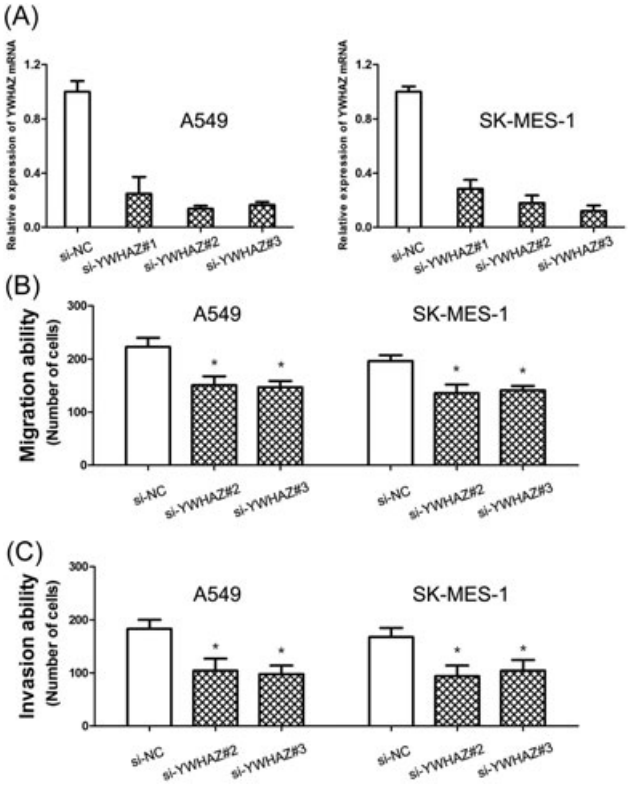

FIGURE 4 YWHAZ negatively regulates NSCLC cell migration and invasion. A, The efficiency of si-YWHAZs is confirmed by qRT-PCR in A549 and SK-MES-1 cells. B, Silencing of YWHAZ expression represses cell migration in A549 and SK-MES-1 cells. C, Silencing of YWHAZ expression inhibits cell invasion in A549 and SK-MES-1 cells. (*$P < 0.001$). NSCLC, non-small–cell lung cancer; qRT-PCR, quantitative real-time polymerase chain reaction

venous and lymphatic invasion, and poor pathological stage in patients with gastric cancer. Meanwhile, Watanabe et al[24] revealed that levels of YWHAZ expression were associated with higher rate of lymph node metastasis, larger tumor size, and poorer Siewert type in patients with adenocarcinoma of the esophagogastric junction. In tongue squamous cell carcinoma patients, YWHAZ overexpression was suggested to be associated with T stage and lymph node metastasis.[25] In prostate cancer patients, high levels of YWHAZ expression were correlated with high Gleason score, PSA relapse and castration-resistant.[26] In addition, the association between high YWHAZ expression and clinical progression was also observed in ovarian cancer[14] and hepatocellular carcinoma.[27]

In our study, we assessed the prognostic value of YWHAZ in NSCLC patients through analyzing the TCGA database, and found YWHAZ expression was negatively correlated with overall survival time in lung adenocarcinoma patients, but not significantly associated overall survival time in lung squamous cell carcinoma patients. However, high levels of YWHAZ protein expression was significantly associated with short overall survival time in lung adenocarcinoma patients and lung squamous cell carcinoma patients from our cohort. In lung squamous cell carcinoma patients, Zhao et al[22] found patients with high expression of YWHAZ had shorter overall survival and disease-free survival than patients with low-expression of YWHAZ. In lung adenocarcinoma patients, Tong et al[20] and Xue et al[28] consistently showed YWHAZ overexpression was significantly associated with unfavorable prognosis. Moreover, Zhao et al[22] also reported NSCLC patients with high-expression of YWHAZ had poor overall survival than patients with low-expression of YWHAZ. Therefore, YWHAZ overexpression was a credible unfavorable prognostic factor for NSCLC patients. In addition, YWHAZ overexpression was also found to serve as a predictor for poor prognosis in ovarian cancer,[14] gastric cancer,[23] tongue squamous cell carcinoma,[25] prostate cancer,[26] and breast cancer.[29,30] However, no correlation between YWHAZ expression and prognosis was observed in bladder cancer patients[31] and hepatocellular carcinoma patients.[32]

The above result showed that YWHAZ expression was associated with lymph node metastasis and present distant metastasis in NSCLC patients, and several published reports showed YWHAZ played important role in regulating tumor cell metastasis.[33,34] Thus, we conducted the loss-of-function study to explore the effect of YWHAZ on NSCLC cell migration and invasion, and silencing of YWHAZ expression repressed NSCLC cell migration and invasion. In addition, basic studies indicated YWHAZ was modulated by microRNAs, and mediated signaling pathways to regulate tumor cell migration and invasion.[35-39] Therefore, we will further try to explore the molecular mechanism of YWHAZ in NSCLC cell at following studies.

In conclusion, YWHAZ is overexpressed in NSCLC tissues, and associated with the malignant status and unfavorable prognosis in NSCLC patient. Silencing of YWHAZ expression represses NSCLC cell migration and invasion.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest.

ORCID

Jiangang Ma http://orcid.org/0000-0003-0921-1288

REFERENCES

1. Chen W, Sun K, Zheng R, et al. Cancer incidence and mortality in china, 2014. *Chinese Journal of Cancer Research*. 2018;30:1-12.
2. Minguet J, Smith KH, Bramlage P. Targeted therapies for treatment of non-small cell lung cancer-recent advances and future perspectives. *Int J Cancer*. 2016;138:2549-2561.
3. Chen W, Zheng R, Zhang S, et al. Cancer incidence and mortality in china, 2013. *Cancer Lett*. 2017;401:63-71.
4. Xu J, Acharya S, Sahin O, et al. Deficiency of 14-3-3ε and 14-3-3ζ by the wnt1 promoter-driven cre recombinase results in pigmentation defects. *BMC Res Notes*. 2016;9:180-192.
5. Kasinski A, Dong X, Khuri FR, Boss J, Fu H. Transcriptional regulation of YWHAZ, the gene encoding 14-3-3ζ . *PLOS One*. 2014;9:e93480.
6. Zhu G, Fan Z, Ding M, et al. An EGFR/PI3K/AKT axis promotes accumulation of the Rac1-GEF tiam1 that is critical in EGFR-driven tumorigenesis. *Oncogene*. 2015;34:5971-5982.
7. Cheng L, Wang P, Yang S, et al. Identification of genes with a correlation between copy number and expression in gastric cancer. *BMC Med Genomics*. 2012;5:14-553.
8. Wang W, Zhang L, Wang Y, et al. Involvement of miR-451 in resistance to paclitaxel by regulating YWHAZ in breast cancer. *Cell Death & Disease*. 2017;8:e3071-e16081.
9. Tang Y, Lv P, Sun Z, et al. Identification of stromal differentially expressed proteins in the colon carcinoma by quantitative proteomics. *Electrophoresis*. 2013;34:1679-1692.
10. Liu M, Liu X, Ren P, et al. A cancer-related protein 14-3-3ζ is a potential tumor-associated antigen in immunodiagnosis of hepatocellular carcinoma. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. *Tumor Biol*. 2014;35:4247-4256.
11. Song T, Tian X, Kai F, et al. Expression profiles for 14-3-3ζ and CCL20 in pancreatic cancer and chronic pancreatitis. *Pathol Res Pract*. 2014;210:335-341.
12. Murata T, Takayama K, Urano T, et al. 14-3-3ζ, a novel androgen-responsive gene, is upregulated in prostate cancer and promotes prostate cancer cell proliferation and survival. *Clin Cancer Res*. 2012;18:5617-5627.
13. Liu S, Wu Y, Yang T, Feng C, Jiang H. Coexistence of YWHAZ amplification predicts better prognosis in muscle-invasive bladder cancer with CDKN2A or TP53 loss. *Oncotarget*. 2016;7:34752-34758.
14. Kim HJ, Sung SH, Kim CY, et al. 14-3-3ζ overexpression is associated with poor prognosis in ovarian cancer. *Yonsei Med J*. 2018;59:51-56.
15. Tan SC, Ismail MP, Duski DR, Othman NH, Bhavaraju VMK, Ankathil R. Identification of optimal reference genes for normalization of RT-qPCR data in cancerous and non-cancerous tissues of human uterine cervix. *Cancer Invest*. 2017;35:163-173.
16. Han X, Han Y, Jiao H, Jie Y. 14-3-3ζ regulates immune response through stat3 signaling in oral squamous cell carcinoma. *Mol Cells*. 2015;38:112-121.
17. Peyvandi H, Peyvandi AA, Safaei A, Zamanian Azodi M, Rezaei-Tavirani M. Introducing potential key proteins and pathways in human laryngeal cancer: a system biology approach. *Iranian Journal of Pharmaceutical Research*. 2018;17:415-425.
18. Janssen JJWM, Klaver SM, Waisfisz Q, et al. Identification of genes potentially involved in disease transformation of CML. *Leukemia*. 2005;19:998-1004.
19. Zhan C, Zhang Y, Ma J, et al. Identification of reference genes for qRT-PCR in human lung squamous-cell carcinoma by RNA-Seq. *Acta Biochim Biophys Sin*. 2014;46:330-337.
20. Tong S, Xia T, Fan K, et al. 14-3-3ζ promotes lung cancer cell invasion by increasing the snail protein expression through atypical protein kinase c (aPKC)/NF-κB signaling. *Exp Cell Res*. 2016;348:1-9.
21. Chen Z, Liu J, Lin L, et al. Analysis of differentially expressed proteome in urine from non-small cell lung cancer patients. *Zhongguo Fei Ai Za Zhi*. 2015;18:138-145.
22. Zhao Y, Qiao W, Wang X, et al. 14-3-3ζ/TGFβR1 promotes tumor metastasis in lung squamous cell carcinoma. *Oncotarget*. 2016;7:82972-82984.
23. Nishimura Y, Komatsu S, Ichikawa D, et al. Overexpression of YWHAZ relates to tumor cell proliferation and malignant outcome of gastric carcinoma. *Br J Cancer*. 2013;108:1324-1331.
24. Watanabe N, Komatsu S, Ichikawa D, et al. Overexpression of YWHAZ as an independent prognostic factor in adenocarcinoma of the esophago-gastric junction. *Am J Cancer Res*. 2016;6:2729-2736.
25. Jin LM, Han XH, Jie YQ, Meng SS. 14-3-3ζ silencing retards tongue squamous cell carcinoma progression by inhibiting cell survival and migration. *Cancer Gene Ther*. 2016;23:206-213.
26. Rüenauver K, Menon R, Svensson MA, et al. Prognostic significance of YWHAZ expression in localized prostate cancer. *Prostate Cancer Prostatic Dis*. 2014;17:310-314.
27. Chen M, Hu W, Xiong CL, et al. MiR-22 targets YWHAZ to inhibit metastasis of hepatocellular carcinoma and its down-regulation predicts a poor survival. *Oncotarget*. 2016;7:80751-80764.
28. Xue M, Tao W. Upregulation of MUC1 by its novel activator 14-3-3ζ promotes tumor invasion and indicates poor prognosis in lung adenocarcinoma. *Oncol Rep*. 2017;38:2637-2646.
29. Xu J, Acharya S, Sahin O, et al. 14-3-3ζ turns TGF-β's function from tumor suppressor to metastasis promoter in breast cancer by contextual changes of smad partners from p53 to Gli2. *Cancer Cell*. 2015;27:177-192.
30. Chang CC, Zhang C, Zhang Q, et al. Upregulation of lactate dehydrogenase a by 14-3-3zeta leads to increased glycolysis critical for breast cancer initiation and progression. *Oncotarget*. 2016;7:35270-35283.
31. Liu S, Jiang H, Wen H, Ding Q, Feng C. Knockdown of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) enhances tumorigenesis both in vivo and in vitro in bladder cancer. *Oncol Rep*. 2018;39:2127-2135.
32. Zhao JF, Zhao Q, Hu H, et al. The ASH1-miR-375-YWHAZ signaling axis regulates tumor properties in hepatocellular carcinoma. *Mol Ther Nucleic Acids*. 2018;11:538-553.
33. Raychaudhuri K, Chaudhary N, Gurjar M, et al. 14-3-3σ gene loss leads to activation of the epithelial to mesenchymal transition due to the stabilization of c-Jun protein. *J Biol Chem*. 2016;291:16068-16081.
34. Tang Y, Lv P, Sun Z, Han L, Luo B, Zhou W. 14-3-3ζ up-regulates hypoxia-inducible factor-1α in hepatocellular carcinoma via activation of PI3K/AKT/NF-κB signal transduction pathway. *Int J Clin Exp Pathol*. 2015;8:15845-15853.

35. Zhang X, Xing ND, Lai CJ, et al. MicroRNA-375 suppresses the tumor aggressive phenotypes of clear cell renal cell carcinomas through regulating YWHAZ. *Chin Med J*. 2018;131:1944-1950.
36. Song T, Tian X, Kai F, et al. Loss of Par3 promotes lung adenocarcinoma metastasis through 14-3-3ζ protein. *Oncotarget*. 2016;7:64260-64273.
37. Mao L, Zhang Y, Deng X, Mo W, Yu Y, Lu H. Transcription factor KLF4 regulates microRNA-544 that targets YWHAZ in cervical cancer. *Am J Cancer Res*. 2015;5:1939-1953.
38. Li Y, Wang J, Dai X, et al. MiR-451 regulates FOXO3 nuclear accumulation through YWHAZ in human colorectal cancer. *Am J Transl Res*. 2015;7:2775-2785.
39. Chen CH, Chuang SM, Yang MF, Liao JW, Yu SL, Chen JJW. A novel function of YWHAZ/β-catenin axis in promoting epithelial-mesenchymal transition and lung cancer metastasis. *Molecular Cancer Research*. 2012;10:1319-1331.

How to cite this article: Deng Y, Zheng J, Ma J. The clinical and prognostic significance of YWHAZ in non-small–cell lung cancer patients: Immunohistochemical analysis. *J Cell Biochem*. 2018;1-9. https://doi.org/10.1002/jcb.27915