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P4HA1 expression and function in esophageal squamous cell carcinoma

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Abstract

This study aimed to explore the effect of P4HA1 (prolyl 4-hydroxylase subunit α1) and its ratio on the prognosis of esophageal squamous cell carcinoma. The expression data of P4HA1 in esophageal cancer in The Cancer Genome Atlas and Genotype-Tissue Expression were collected using the public database gene expression profiling interactive analysis. The expression levels of P4HA1 were examined by immunohistochemistry. The relationship between P4HA1 expression and clinicopathological parameters was analyzed the χ^2 test. Survival analysis was performed to investigate the effect of P4HA1 and its ratio on prognosis. Compared with normal esophageal mucosal epithelium, there was higher P4HA1 gene mRNA in esophageal cancer tissue. Regarding the expression level, no significant difference was observed in patients with stage I–IV esophageal cancer. Immunohistochemistry showed that P4HA1 was highly expressed in esophageal squamous cell carcinoma (68.7%), while it was negatively expressed in paracancerous tissues. There was a significant difference in expression between cancer and adjacent tissues. The expression of P4HA1 associated with the degree of tumor differentiation, site, lymph node metastasis, and tumor node metastasis stage. The prognostic factors that affected the OS (overall survival) of esophageal cancer patients were the degree of differentiation, lymph node metastasis, and P4HA1 expression. Multivariate analysis of the OS results of patients showed that lymph node metastases and P4HA1 expression were independent prognostic factors that affected the OS of esophageal cancer patients. The prognostic factors affecting the PFS (progression-free survival) of esophageal cancer patients in the univariate survival analysis were as follows: degree of differentiation, lymph node metastasis, and P4HA1 expression. In addition, multivariate analysis of the PFS results of patients showed that lymph node metastasis and P4HA1 expression were independent prognostic factors that affected the PFS of esophageal cancer patients. P4HA1 may be a novel potential biomarker for the early diagnosis, prognosis, and targeted therapy of esophageal cancer.

Abbreviations: CI = confidence interval, ESCC = esophageal squamous cell carcinoma, GEPIA = gene expression profiling interactive analysis, HR = hazard ratio, OS = overall survival, P4HA1 = prolyl 4-hydroxylase subunit α1, PFS = progression-free survival, PPI = protein–protein interaction, TMA = tissue microarray, TNM = tumor node metastasis.

Keywords: ESCC, immunohistochemistry, P4HA1, prognosis, tissue microarray

1. Introduction

Esophageal cancer is one of the most common malignancies worldwide, ranking ninth and sixth in incidence and mortality, respectively.[1] Owing to the nonspecific symptoms of esophageal cancer, affected patients often already have metastases on first presentation, and their survival rate is low.[2] Esophageal cancer has long been a major malignancy that threatens the health of Chinese citizens, with incidence and mortality rates respectively ranking third and fourth among all malignancies in China.[3] However, the incidence of esophageal cancer shows significant regional differences, and the incidence of esophageal squamous cell carcinoma (ESCC) is higher than that of esophageal adenocarcinomas. Although major breakthroughs have been achieved in the diagnosis and treatment of esophageal cancer in China in recent years, the post-treatment recurrence rate is still high, and there are limitations in the available drugs and treatment strategies following metastasis. Therefore, the overall survival (OS) of patients with ESCC in China remains unclear. There is also a lack of reliable prognostic markers for therapeutic guidance. Prolyl 4-hydroxylase subunit α1 (P4HA1) promotes collagen synthesis and accelerates cancer fibrosis,[4] which in turn promotes metastasis. *P4HA1* has been reported to be

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The datasets generated during and/or analyzed during the current study are publicly available.

EC tissues and normal adjacent tissues were collected from 120 patients diagnosed with EC who underwent surgical resection at The First Affiliated Hospital of Xinjiang Medical University. This study was reviewed by the Ethics Committee of the First Affiliated Hospital, Xinjiang Medical University (SL-2022-006), and all participants provided written informed consent.

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significantly upregulated in lung adenocarcinoma, pan-cancer, and breast cancer, indicating that it can be used as a prognostic biomarker for cancer.[5] However, the mechanism of action of *P4HA1* in ESCC remains unclear.

2. Materials and methods

2.1. Expression of the P4HA1 gene in cancerous and healthy esophageal tissues

Basic clinical data of tumor patients, such as demographic data, treatment regimen, clinical stage, tumor pathology, survival status, and other details such as messenger RNA, microRNA, copy number, mutations, protein, and methylation information, were obtained from The Cancer Genome Atlas (http://cancergenome.nih.gov/) and Genotype-Tissue Expression (https://www.gtexportal.org) databases. Gene expression profiling interactive analysis (GEPIA) was conducted on both ESCC and healthy tissues.

2.2. Expression status in different ESCC stages

The GEPIA database was used to evaluate P4HA1 expression in different stages in ESCC.

2.3. Survival analysis

The GEPIA database was used to analyze the relationship between P4HA1 expression levels and ESCC patient outcomes.

2.4. Protein–protein interaction (PPI) network

The STRING database was used to analyze the *P4HA1* PPI network and the GEPIA database was used to obtain a scatterplot of related genes.

2.5. Tissue microarray (TMA)

A TMA was constructed by the Pathology Department of the First Affiliated Hospital of Xinjiang Medical University, and technical support was provided by Shanghai Oukuo Biotechnology Co. Ltd (Shanghai, China). The most representative cancerous and paracancerous regions in 240 healthy mucosal paraffin blocks were used to construct a TMA.

2.6. General patient information

In this study, we retrospectively analyzed squamous cell carcinoma specimens from 237 patients who were diagnosed and underwent surgery at the First Affiliated Hospital of Xinjiang Medical University between March 2008 and December 2017. In total, there were 120 Han Chinese and 117 Kazakhs. The general patient characteristics are shown in Table 1. Histological specimens were used for hematoxylin and eosin sectioning and immunohistochemical staining. Clinicopathological data were used to analyze the correlation between P4HA1 expression and clinicopathological markers. Telephone calls or medical record reviews were used for follow-up, which ended in June 2020. OS and progression-free survival (PFS) ranged from 1 to 104 months. The median OS was 32 months and the median PFS was 22 months.

This study was approved by the ethics committee of the Hainan Wanning People Hospital, and written informed consent was obtained from all participants before enrollment in this study.

2.7. Immunohistochemistry

A rabbit monoclonal anti-P4HA1 antibody (12658-1-AP; Proteintech Group) was used at a dilution ratio of 1:200. The TMA slides were cleared in xylene and dehydrated using an ethanol gradient. Antigen retrieval was carried out using citrate buffer with a pH of 6.0. Then, 3% hydrogen peroxide was added to inactivate intrinsic peroxidases, and the sections were incubated at room temperature. Subsequently, TMA slides were incubated with the rabbit P4HA1 antibody (1:200) at 4°C overnight. After the slides were washed, a secondary antibody was added and incubated at room temperature. Finally, we performed 3,3′-diaminobenzidine staining, followed by counterstaining, dehydration, and mounting.

Two deputy chief pathologists assessed all immunohistochemical sections in a double-blind manner and calculated the percentage of positive cells. The percentage of P4HA1-positive cells relative to the total number of cancer cells was determined using optical microscopy. For each tissue block, 4 to 5 HP fields with cell counts of > 300 cells/field were enumerated. The scoring criteria used during the assessment were as follows: 0 points, <1% of tumor cells were positively stained; 1 point, 1–10% of tumor cells were positively stained; 2 points, 10% to 50% of tum or cells were positively stained; 3 points, 50% to 75% of tumor cells were positively stained; and 4 points, >75% of tumor cells were positively stained. The staining intensity was evaluated by

Table 1

General characteristics of ESCC patients.

Characteristic	N = 237	Percentage (%)
Age (yr)		
>60	84	35.4%
≤60	153	64.5%
Tumor size (cm)		
<3	73	30.8%
≥3	164	69.2%
Gender		
Male	169	71.3%
Female	68	28.7%
Ethnicity		
Han	120	50.6%
Kazakh	117	49.4%
Degree of differentiation		
Well	40	16.9%
Moderate	126	53.2%
Poor	71	29.9%
Lymph node metastasis		
No	157	66.2%
Yes	80	33.8%
Tumor location		
Upper	12	5%
Middle	142	60%
Lower	83	35%
Invasion depth		
Mucosa	7	3%
Muscularis	99	41.8%
Full thickness	131	55.2%
Vascular invasion		
No	194	81.9%
Yes	43	18.1%
Nerve invasion		
No	186	78.5%
Yes	51	21.5%
Hematogenous metastasis		
No	206	86.9%
Yes	31	13.1%
Postoperative treatment		
No	156	65.8%
Yes	81	34.2%
P4HA1		
Low expression	74	31.2%
High expression	163	68.8%

ESCC = esophageal squamous cell carcinoma, P4HA1 = prolyl 4-hydroxylase subunit α1.

comparison with the negative control group. Cells with pale-yellow or brownish-yellow granules in the cytoplasm or nucleus were considered to be positively stained. The scoring criteria used according to this rule were as follows: positive staining or suspicion of positive staining, 0 points; weakly stained cells, 1 point; moderately positive cells, 2 points; and strongly positive cells, 2 points. The percentage of positively stained tumor cells and staining intensity were summed. For P4HA1, we concluded that there was high expression when the total score was > 1 point; otherwise, expression was defined as low.

2.8. Statistical analysis

SPSS version 25.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. The chi-squared test was used to analyze the correlation between P4HA1 levels and clinicopathological characteristics. Kaplan–Meier curves were plotted for OS analysis. A Cox proportional hazards model was used to confirm independent prognostic factors for OS and PFS. Differences with $P < .05$ were considered statistically significant.

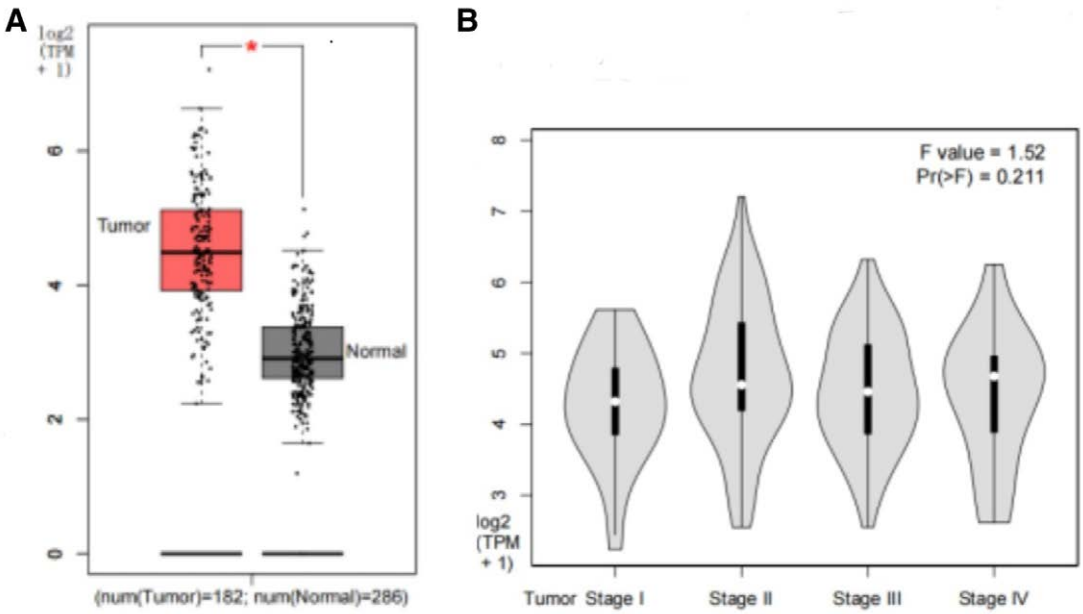

Figure 1. (A) GEPIA database analysis of the expression of P4HA1 in tumor tissues and normal esophageal tissues. (B) Relationship between P4HA1 expression levels and tumor stage. GEPIA = gene expression profiling interactive analysis. P4HA1 = prolyl 4-hydroxylase subunit α1.

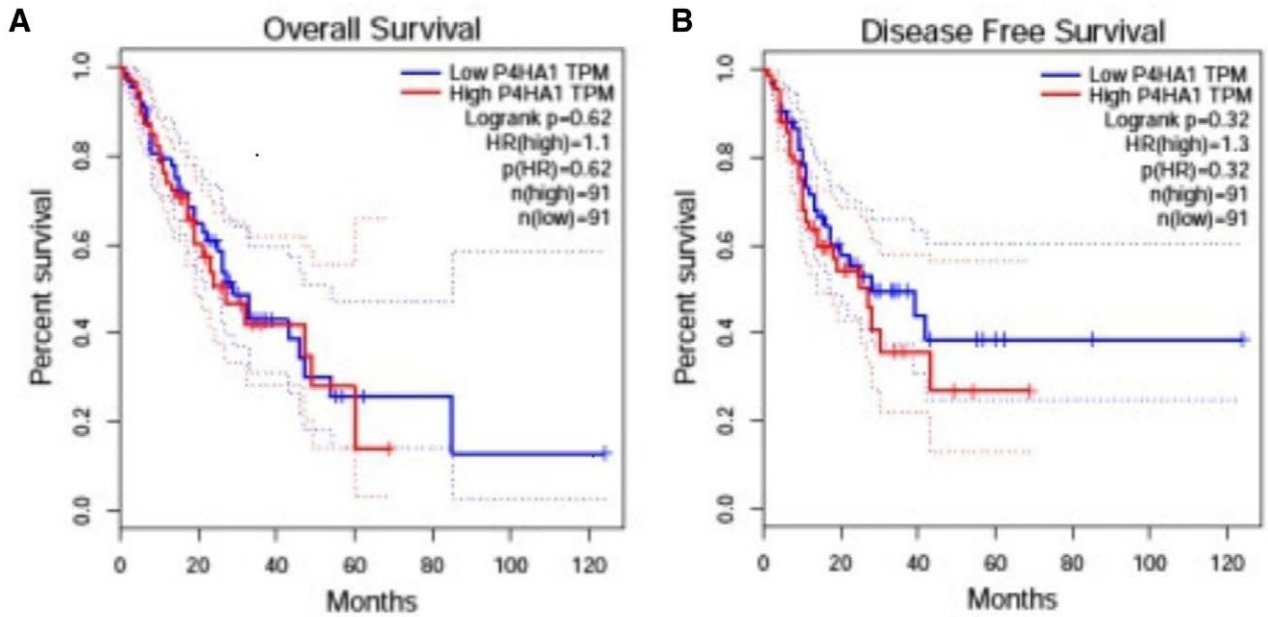

Figure 2. (A) GEPIA database analysis of the relationship between P4HA1 expression levels and the OS of esophageal cancer patients. (B) GEPIA database analysis of P4HA1 expression levels and the PFS of esophageal cancer patients. GEPIA = gene expression profiling interactive analysis. P4HA1 = prolyl 4-hydroxylase subunit α1, PFS = progression-free survival.

3. Results

3.1. P4HA1 expression differences between ESCC tissues and healthy esophageal tissues

To assess differences of P4HA1 expression, GEPIA database analysis was performed. Figure 1A compares the P4HA1 mRNA expression levels in 286 ESCC tissues and 182 healthy esophageal tissues. P4HA1 expression was significantly higher in ESCC tissues than in healthy esophageal tissues ($P < .01$). Figure 1B compares the relationship between P4HA1 expression and clinical stage of ESCC. The results showed that the difference was not statistically significant ($P > .05$).

The GEPIA database was used to assess the relationship of P4HA1 expression levels with OS and PFS in 182 patients with ESCC. The patients were divided into a P4HA1 high expression group (n = 91) and a P4HA1 low expression group (n = 91). The results showed no statistically significant differences between the groups (Fig. 2A and B).

3.2. P4HA1 PPI network and functional analysis

The STRING database was used to analyze the PPI network. Ten P4HA1-interacting proteins were selected as follows: COL3A1, COL1A1, COL5A1, COL4A1, COL1A2, COL13A1, P4HB, LEPRE1 (P3H1), LEPREL1 (P3H2), and LEPREL2 (P3H3). Figure 3 shows the interaction network, and Figure 4 presents the scatterplot of P4HA1 and other genes.

3.3. Clinicopathological characteristics

Table 1 shows the clinicopathological characteristics of the 237 enrolled ESCC patients. The median age at diagnosis was 63 years (35–87) and the mean follow-up period was 28 months (1–104). A total of 176 patients (74.2%) died during the follow-up period.

3.4. Expression of P4HA1 in ESCC and healthy esophageal tissues

We used immunohistochemistry to measure the expression of P4HA1 in the esophageal mucosal epithelium and ESCC tissues. Positive P4HA1 expression was localized in the cytoplasm of tumor cells and appeared as brown granules (Fig. 5). The P4HA1 positivity rate in esophageal cancer tissues was 68.7% (163/237), while para-cancerous tissues had low P4HA1 expression (Table 2). P4HA1 expression was significantly increased in esophageal cancer ($P < .001$).

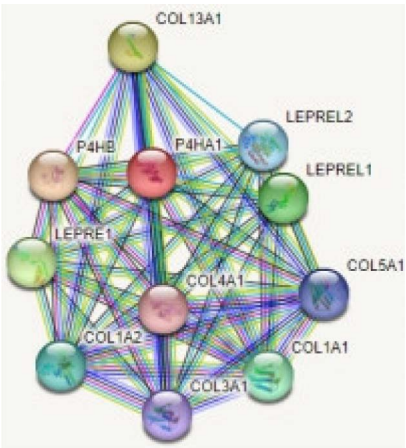

Figure 3. Protein–protein interaction (PPI) network of P4HA1. P4HA1 = prolyl 4-hydroxylase subunit α1.

3.5. P4HA1 expression in esophageal cancer and para-cancerous tissues and its relationship with clinicopathological parameters and prognosis

A comparison of the relationship between P4HA1 expression in esophageal cancer and clinicopathological parameters revealed that P4HA1 expression was associated with the degree of tumor differentiation ($P = .001$), site ($P = .042$), lymph node metastases ($P = .001$), and tumor node metastasis (TNM) stage ($P = .048$). By contrast, P4HA1 expression was not associated with age ($P = .947$), sex ($P = .489$), race ($P = .125$), tumor size ($P = .114$), invasion depth ($P = .790$), lymphovascular invasion ($P = .213$), neural invasion ($P = .979$), hematogenous metastasis ($P = .126$), or radiochemotherapy ($P = .498$). The details are presented in Table 3.

3.6. Relationship between P4HA1 expression and the prognosis of esophageal cancer patients

Four of the 237 enrolled patients with esophageal cancer were lost to follow-up. Kaplan–Meier estimation was used for univariate survival analysis. The results showed that the prognostic factors that affect the OS of esophageal cancer patients (Table 4 and Fig. 6A–C) included the degree of differentiation ($P < .001$), lymph node metastasis ($P < .001$), and P4HA1 expression ($P < .001$). Multivariate analysis (Cox proportional hazards model) of the OS results of patients showed that lymph node metastases (hazard ratio [HR], 1.677; 95% confidence interval [CI], 1.146–2.454; $P = .001$) and P4HA1 expression (HR, 2.234; 95% CI, 1.310–3.810; $P = .001$) were independent prognostic factors that affected the OS of esophageal cancer patients (Table 5).

The prognostic factors affecting the PFS of esophageal cancer patients (Table 4 and Fig. 6D–F) in the univariate survival analysis included degree of differentiation ($P = .026$), lymph node metastasis ($P < .001$), and P4HA1 expression ($P < .001$). In addition, multivariate analysis (Cox proportional hazards model) of the PFS results of patients showed that lymph node metastasis (HR, 1.753; 95% CI, 1.198–2.564; $P = .004$) and P4HA1 expression (HR, 2.342; 95% CI, 1.378–3.980; $P = .002$) were independent prognostic factors that affected the PFS of esophageal cancer patients (Table 6).

4. Discussion

P4HA1, first discovered in zebrafish, is a highly conserved extracellular matrix protein that mainly accumulates in the basal lamina of the brain. In addition, *P4HA1* promotes the growth of hippocampal neurons during embryonic development.[6–8] Three P4HA subunit isoforms (*P4HA1*, *P4HA2*, and *P4HA3*) can bind P4HB in A2B2 stoichiometry to produce P4H1, P4H2, and P4H3 tetramers, respectively. P4H hydroxylates proline residues to form 4-hydroxylproline, which is vital for the post-translational modification of collagen.[9–11] *P4HA1* can regulate collagen synthesis and secretion in fibroblasts, thereby altering the extracellular matrix composition of cells, thus affecting tumor adhesion and migration.[12] In this study, the STRING database was used to analyze the PPI network of P4HA1. The results of this investigation showed that *P4HA1* was intimately associated with the *COL3A1*, *COL1A1*, *COL5A1*, *COL4A1*, *COL1A2*, and *COL13A1* genes.

At present, there are few studies on *P4HA1* in tumors, and existing studies have mainly assessed the roles of *P4HA1* and tumor proliferation, metastasis, and immune cell infiltration.[13–15] Agarwal et al[13] found that *P4HA1* promotes colorectal cancer metastasis while its downregulation decreases the distal spread of tumor cells. Diethyl-pythiDC, a small-molecule inhibitor of P4HA1, inhibits colorectal cancer

growth. In a patient-derived xenograft model of colorectal cancer, diethyl-pythiDC decreased tumor growth. Cao et al[14] conducted a study on pancreatic cancer and found that *P4HA1* silencing significantly inhibited the proliferation, chemoresistance, and stemness of pancreatic ductal adenocarcinomas cells. P4HA1-HIF1 is a critical regulatory factor of glycolysis and pancreatic ductal adenocarcinomas carcinogenicity, as well as a potential target for pancreatic cancer treatment. Thorsson et al[15] found that P4HA1 is positively correlated with TMA infiltration in many tumor models, including lung

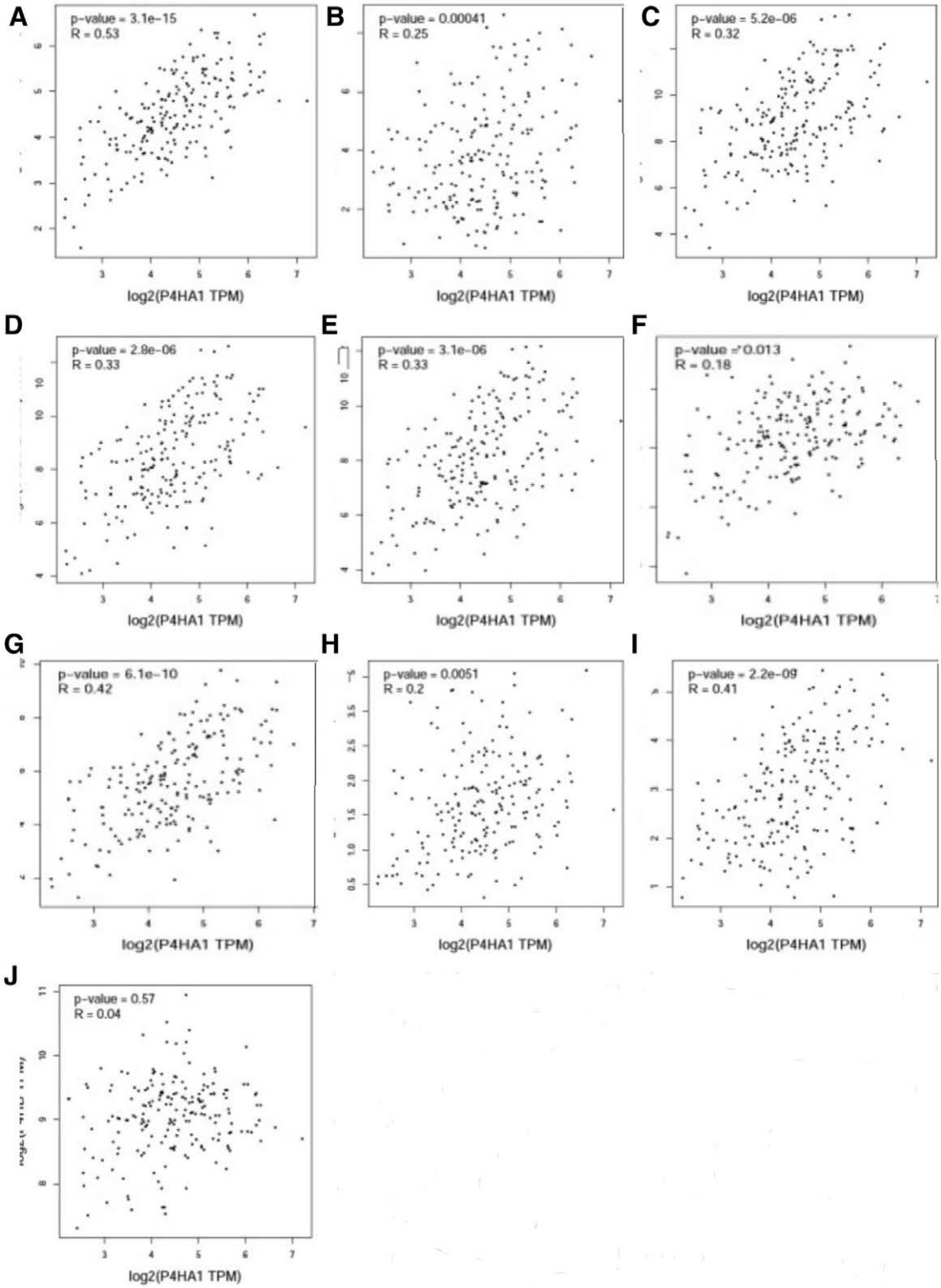

Figure 4. Scatter map between P4HA1 and other genes. (A) Scatter map of P4HA1 and P3H1 genes; (B) scatter map of P4HA1 and P3H2 genes; (C) scatter map of P4HA1 and COL1A1 genes; (D) scatter map of P4HA1 and COL1A2 genes; (E) scatter map of P4HA1 and COL3A1 genes; (F) scatter map of P4HA1 and COL4A1 genes; (G) scatter map of P4HA1 and COL5A1 genes; (H) scatter map of P4HA1 and COL13A1 genes; (I) scatter map of P4HA1 and P3H3 genes; (J) scatter map of P4HA1 and P4HB genes. P4HA1 = prolyl 4-hydroxylase subunit α1.

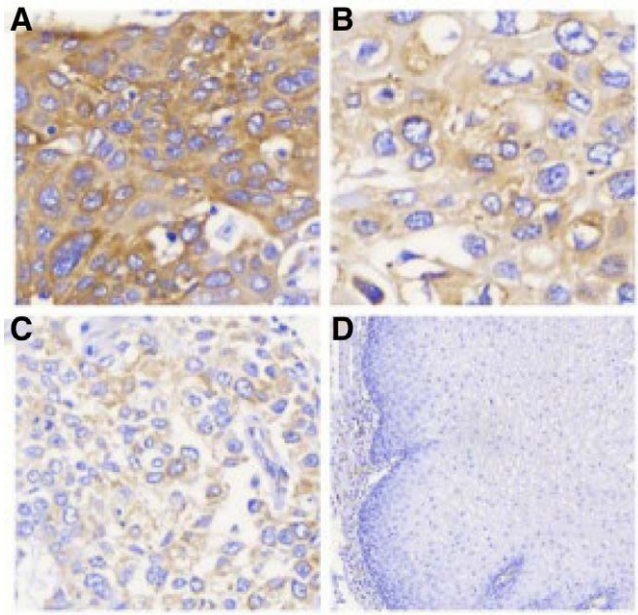

Figure 5. Expression of P4HA1 in squamous cell carcinoma and paracancer tissues. (A) Strong positive expression of P4HA1 in esophageal squamous cell carcinoma. (B) Moderate intensity positive expression of P4HA1 in esophageal squamous cell carcinoma. (C) Weak positive expression of P4HA1 in esophageal squamous cell carcinoma. (D) P4HA1 was not expressed in normal squamous epithelium. P4HA1 = prolyl 4-hydroxylase subunit α1.

adenocarcinoma, but is negatively correlated with cytotoxic immune cells. A similar phenomenon was observed when data from the ImmuCellAI database were used. At the same time, it was found that P4HA1 expression was positively correlated with tumor mutation load and microsatellite instability in different cancers.

In this study, the P4HA1 positivity rate of esophageal cancer tissues was 68.7% (163/237), while para-cancerous tissues did not show detectable P4HA1 expression. P4HA1 expression was significantly increased in esophageal carcinoma. These results were consistent with the GEPIA database analysis results. A comparison of the relationship between P4HA1 expression levels in esophageal cancer and clinicopathological parameters revealed that P4HA1 expression is associated with the degree of tumor differentiation, site, lymph node metastases, and TNM stage. A study on head and neck squamous cell carcinoma by Li et al[16] found that

Table 2

Expression of the *P4HA1* gene in ESCC and healthy esophageal tissues.

	P4HA1			
	Low expression	High expression	Sum	*P*
Cancer	74	163	237	.000
Normal	237	0	237	
Sum	311	163	474	

ESCC = esophageal squamous cell carcinoma, P4HA1 = prolyl 4-hydroxylase subunit α1.

Table 3

Expression level of P4HA1 in ESCC and its relationship with clinicopathologic parameters.

		P4HA1			
Clinicopathological characteristics		Low expression (n = 74)	High expression (n = 163)	χ^2	*P*
Age	≤60	26	58	0.004	.947
	>60	48	105		
Gender	Male	55	114	0.478	.489
	Female	19	49		
Ethnicity	Han Chinese	32	88	2.351	.125
	Kazakhs	42	75		
Degree of differentiation	High	22	18	14.973	.001
	Moderate	38	88		
	Low	14	57		
Site	Upper segment	2	10	6.328	.042
	Middle segment	53	89		
	Lower segment	19	64		
Tumor size	<3 cm	28	45	2.499	.114
	≥3 cm	46	118		
Lymph node metastases	Present	6	74	31.650	.001
	Absent	68	89		
TNM staging	T1	11	8	7.892	.048
	T2	39	93		
	T3	16	48		
	T4	8	14		
Invasion depth	Mucosal layer	3	4	0.471	.790
	Muscle layer	31	68		
	All layers	40	91		
Lymphovascular invasion	Present	10	33	1.553	.213
	Absent	64	130		
Neural invasion	Present	16	35	0.01	.979
	Absent	58	128		
Hematogenous metastasis	Present	6	25	2.340	.126
	Absent	68	138		
Radiochemotherapy	Yes	23	58	0.458	.498
	No	51	105		

ESCC = esophageal squamous cell carcinoma, P4HA1 = prolyl 4-hydroxylase subunit α1.

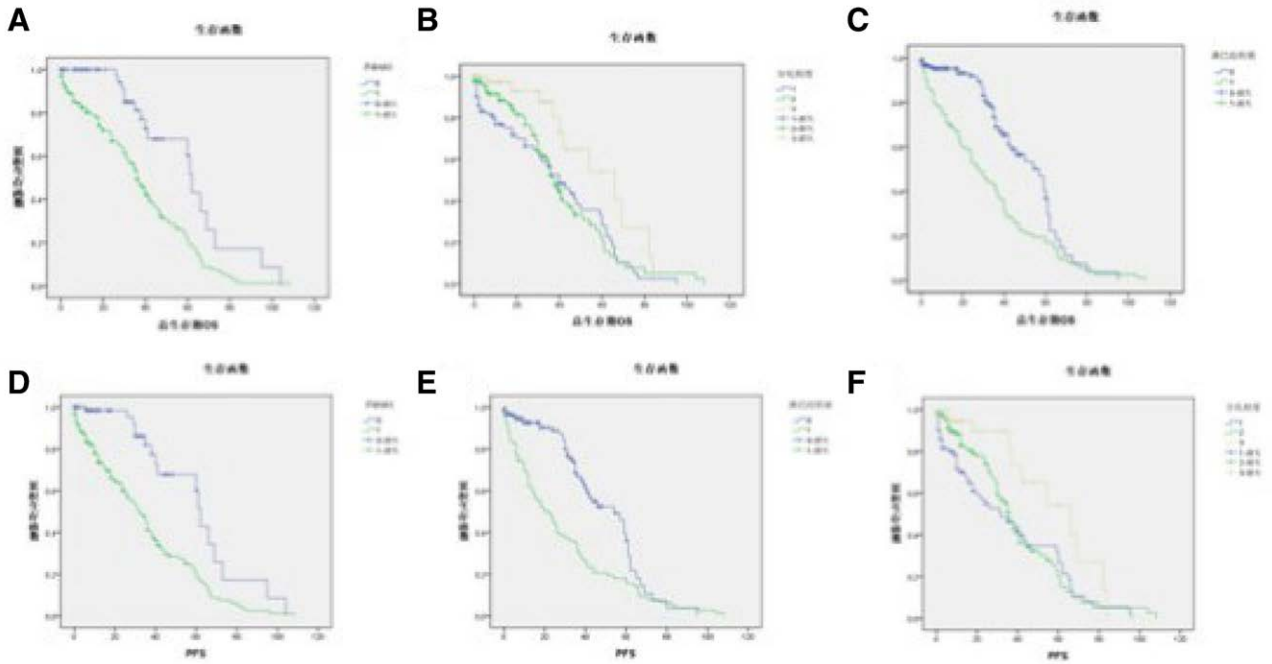

Figure 6. Kaplan–Meier survival analysis. (A) Overall survival (OS) of patients with ESCC expressing *P4HA1*. (B) OS of patients with ESCC of different degrees of differentiation. (C) OS of patients with ESCC and lymph node metastasis. (D) Progression-free survival (PFS) of patients with ESCC expressing *P4HA1*. (E) PFS of patients with ESCC and lymph node metastasis. (F) PFS of patients with ESCC of different degrees of differentiation. ESCC = esophageal squamous cell carcinoma. P4HA1 = prolyl 4-hydroxylase subunit α1.

Table 4

Univariate analysis of factors related to OS and PFS in ESCC patients.

	OS		PFS	
Characteristic	χ^2	***P* value**	χ^2	***P* value**
Gender (female vs male)	0.292	.589	0.473	.491
Age (≤60 vs > 60)	0.931	.335	1.357	.244
Ethnicity (Han vs Kazakh)	0.007	.934	0.232	.630
Tumor location (upper vs middle vs lower)	0.408	.815	0.237	.888
Tumor size (<3 vs ≥ 3 cm)	0.057	.811	0.071	.789
Degree of differentiation (PD vs MD vs WD)	7.117	.028	7.293	.026
Invasion depth (MA vs MS vs FT)	1.590	.452	1.913	.384
Lymph node metastasis (no vs yes)	16.328	.000	18.869	.000
Vascular invasion (no vs yes)	1.575	.209	1.609	.205
Nerve invasion (no vs yes)	1.198	.274	0.457	.499
Hematogenous metastasis (no vs yes)	0.176	.675	0.464	.496
Postoperative treatment (no vs Che + Ra)	0.874	.350	0.463	.496
P4HA1 expression (negative expression vs positive expression)	16.529	.000	19.278	.000
TNM (T1 vs T2 vs T3 vs T4)	3.894	.273	5.837	.120

ESCC = esophageal squamous cell carcinoma, OS = overall survival, P4HA1 = prolyl 4-hydroxylase subunit α1, PFS = progression-free survival, TNM = tumor node metastasis.

P4HA1 mRNA and protein levels were significantly higher in head and neck squamous cell carcinoma tissues than in matched normal tissues. High P4HA1 expression in head and neck squamous cell carcinoma tissues was significantly associated with the tumor type, lymph node metastasis, and pathological stage.

With regard to the relationship between P4HA1 expression and the prognosis of esophageal cancer patients, Kaplan–Meier estimation was used for univariate survival analysis, and the results revealed several prognostic factors that affect the OS of esophageal cancer patients, including the degree of differentiation, lymph node metastasis, and P4HA1 expression. Multivariate analysis also showed that lymph node metastasis and P4HA1 expression were independent prognostic factors affecting the OS of patients with esophageal cancer. Additionally, when Kaplan–Meier estimation was used for univariate survival analysis to reveal the prognostic factors that affect the PFS of esophageal cancer patients, the identified factors included the degree of differentiation, lymph node metastasis, and P4HA1 expression. In addition, multivariate analysis (Cox proportional hazards model) of the PFS results of patients showed that lymph node metastases, P4HA1 expression, and TNM stage were independent prognostic factors that affected the PFS of esophageal cancer patients. Ning et al[17] showed that P4HA1 expression was significantly upregulated in lung squamous cell carcinoma and lung adenocarcinoma tumor tissues. In addition, high P4HA1 expression was found to be associated with a poor prognosis of lung adenocarcinoma, suggesting that P4HA1 can be used as an early diagnostic and prognostic biomarker for patients with these tumors. These previously published findings were in agreement with the results of the present study.

5. Conclusion

Based on the results of this study and the available Chinese and international papers, we speculate that P4HA1 may be a novel potential biomarker for early diagnosis, prognosis, and targeted therapy of esophageal cancer.

Author contributions

Conceptualization: Yongqiang Yang, Wenbin Gou.
Data curation: Beiwen Song.

Table 5

Multivariate analysis of OS in ESCC patients.

Characteristic	*P* value	HR	95.0% CI	
Lymph node metastasis (no vs yes)	.001	1.677	1.146	2.454
P4HA1 expression (low vs high)	.001	2.234	1.310	3.810

CI = confidence interval, ESCC = esophageal squamous cell carcinoma, HR = hazard ratio, OS = overall survival, P4HA1 = prolyl 4-hydroxylase subunit α1.

Table 6

Multivariate analysis of factors related to OS and PFS in ESCC patients.

Characteristic	*P* value	HR	95% CI	
Lymph node metastasis (no vs yes)	.004	1753	1.1498	2.564
P4HA1 expression (low vs high)	.002	2.342	1.378	3.980
TNM (T1 vs T2 vs T3 vs T4)	.046	1.338	1.005	1.781

CI = confidence interval, ESCC = esophageal squamous cell carcinoma, HR = hazard ratio, OS = overall survival, P4HA1 = prolyl 4-hydroxylase subunit α1, PFS = progression-free survival, TNM = tumor node metastasis.

Writing – review & editing: Yongqiang Yang, Wenbin Gou.
Writing – original draft: Wenbin Gou.

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