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Low expression of PDHA1 predicts poor prognosis in gastric cancer

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ABSTRACT

PDH E1 component subunit alpha (PDHA1) has been reported to be biologically significant in several human tumors. The aim of this study was to investigate the expression of PDHA1 in gastric cancer (GC) and its relationship with clinicopathological characteristics and prognosis. Oncomine analysis of neoplastic vs. normal tissue showed that the mRNA levels of *PDHA1* were significantly underexpressed in different types of GC across three analyses. Underexpression of *PDHA1* was found in intestinal-type GC ($P = 0.009$), diffuse-type GC ($P = 0.036$), and mixed-type GC ($P = 0.025$). Immunohistochemical staining of the 174 GC tissue microarray showed that PDHA1 staining is much stronger in normal mucosa than in GC samples ($P = 0.040$). Furthermore, PDHA1 expression levels were found to be significantly lower in 69.05% (87/126) of poorly differentiated GCs as compared to the well or moderately differentiated ones ($P = 0.037$). Intriguingly, PDHA1 expression was significantly correlated with depth of invasion ($P < 0.001$), lymph node metastasis ($P < 0.001$), TNM stage ($P < 0.001$), and nerve invasion ($P = 0.006$). However, it was not correlated with gender, age, Lauren classification, and lymphovascular invasion ($P > 0.05$ for all). Kaplan-Meier analysis revealed that low tumor expression of PDHA1 was significantly correlated with a poorer overall survival in patients with GC (5-year overall survival rates for patients with low vs high PDHA1 expression = 49.8% vs 72.7%, hazard ratio of death from GC = 2.594, 95% CI = 1.527 to 4.408, $P < 0.001$). Multivariate analysis showed that PDHA1 ($P = 0.025$) was an independent predictor of overall survival. These findings are of potential clinical utility and merit further validation.

1. Introduction

Gastric cancer (GC) is a common malignancy, and the third leading cause of cancer-related mortality worldwide [1]. Because detection is frequently made only at an advanced stage, GC is still a major challenge [2]. Thus, a better understanding of the underlying mechanisms that promote the pathogenesis of GC is urgently needed.

Emerging evidence indicates that reprogramming of cellular metabolism is a hallmark of cancer cells [3–5]. Cancer cells take up and exploit much more glucose than normal cells and switch from oxidative phosphorylation to glycolysis metabolism regardless of oxygen availability, a phenomenon called the Warburg effect or aerobic glycolysis [6,7]. Pyruvate dehydrogenase (PDH) is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and links glycolysis with oxidative metabolism [8–10]. PDH E1 component subunit alpha (PDHE1α or PDHA1) is a pivotal component of PDH that transforms pyruvate into acetyl-CoA [11]. Deficiency of PDHA1 leads to mitochondrial dysfunction and promotes glycolysis [12]. It has been reported that the oncoprotein hepatitis B X-interacting protein (HBXIP) enhances glucose metabolism reprogramming via suppressing synthesis of cytochrome c oxidase 2 (SCO2) and PDHA1 in breast cancer, leading to the increase of lactate production and intracellular glucose, which ultimately promotes tumor growth [13]. PDHA1 has also been reported to be associated with better tumor differentiation in esophageal squamous cell carcinoma. Decreased PDHA1 expression predicts a poor prognosis of patients with esophageal squamous cell carcinoma [12]. A recent study indicates that decreased PDHA1 protein expression is correlated with poor prognosis in prostate cancer [14]. Sun et al. [15] report that reduced PDHA1 expression is associated with poor outcome in patients with hepatocellular carcinoma. Recently, Liu et al. [16] report that miR-21-5p-PDHA1 axis is involved in GC glycolysis and progression. However, the correlation between the PDHA1 protein expression and clinicopathological characteristics remains unclear.

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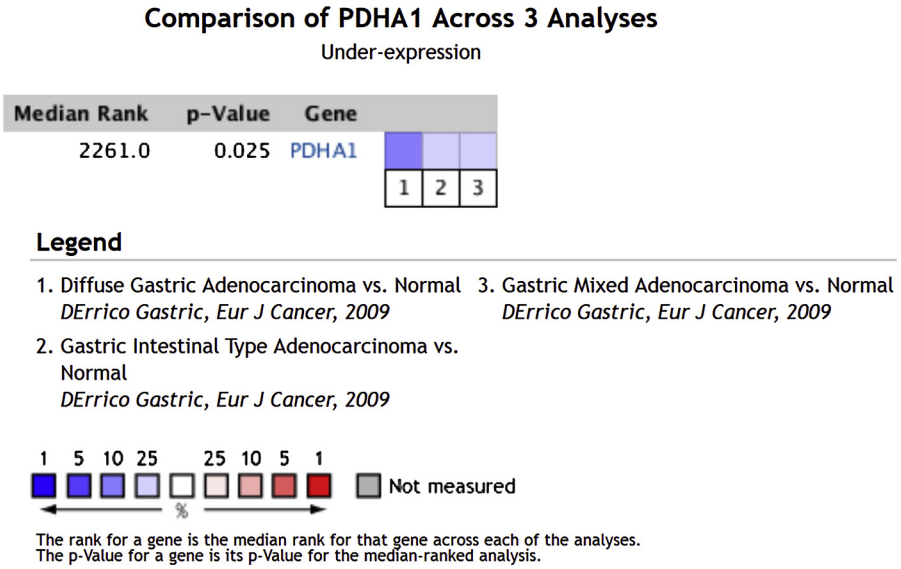

Fig. 1. *PDHA1* expression is decreased in GC tissues in Oncomine database. Oncomine heat map demonstrated a statistically significant decrease in *PDHA1* expression in GC tissues compared with the normal tissues.

The present study investigated the clinicopathological significance of PDHA1 in GCs. The study provides evidence that PDHA1 may serve as a promising prognostic and predictive biomarker for determining malignant properties in patients with GCs.

2. Materials and methods

2.1. Patients and specimens

After approval from the Ethical Review Committee of the Affiliated Tumor Hospital of Nantong University, 174 GC samples and 46 normal gastric mucosa were obtained to construct tissue microarray (TMA). None of the patients received preoperative systemic chemotherapy or radiotherapy. All patients were diagnosed between 10 January 2007 and 25 June 2013. The histological types of GC were classified according to Lauren classification and staged according to the Tumor, Node, Metastasis (TNM) guidelines. The median age at diagnosis of these patients was 63 years (range = 35–83 years), and the median follow-up was 48 months (range = 1–95 months).

2.2. TMA construction and immunohistochemistry

For TMA construction, representative areas were circled on the glass slides and used as a template. The TMA was constructed using a manual tissue puncher/arrayer. For each sample, a 1.0 mm diameter core of tissue was punched from paraffin block. PDHA1 (1:50 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) immunohistochemical staining was performed on a Dako Omnis Autostainer (Dako, Agilent Technologies, Inc., Carpinteria, CA, USA) according to the manufacturer's instructions.

2.3. Assessment of immunohistochemistry

Staining of PDHA1 in TMAs was evaluated independently by two pathologists blinded to all clinical data, by applying a semiquantitative immunoreactivity score (IRS) system that incorporates the percentual area and the intensity of immunoreactivity. The intensity of immunostaining was scored as 0–3 (0, negative; 1, weak; 2, moderate; 3, strong). The percentage of immunoreactive cells was scored as 1 (0–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). Multiplication of the intensity and the percentage resulted in a score ranging from 0 to 12. The optimal cutoff point for the definition of PDHA1 high/low expression subgroups was determined using X-tile software (Yale University). For statistical analysis, cases exhibiting an IRS from 0 to 4 were lumped into the PDHA1 low group, whereas cases with a higher IRS (> 4) were designated PDHA1 high group.

2.4. Oncomine analysis

The expression level of *PDHA1* gene in GC was analyzed using Oncomine database (http://www.oncomine.org). For this, we compared GC vs. normal tissue datasets. Threshold *P*-value of 0.05 was set to obtain data.

2.5. Kaplan-Meier plotter analysis

The prognostic value of the *PDHA1* gene in GC was analyzed using Kaplan-Meier Plotter (http://kmplot.com/analysis/), a database that integrates gene expression data and clinical data. Briefly, by entering the gene symbol, *PDHA1*, the survival curve based on *PDHA1* expression in GC was automatically generated by the Kaplan-Meier plotter analysis tool.

2.6. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Science SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). The associations between PDHA1 expression and clinicopathological characteristics were evaluated by Chi- square (χ^2) test. The probability of differences in overall survival were calculated using the Kaplan-Meier method, with a log-rank test for significance. Cox proportional-hazards regression analysis was used to estimate univariate and multivariate hazard ratios for overall survival. A P value of < 0.05 was deemed statistically significant.

3. Results

3.1. PDHA1 mRNA expression in GC patients

As shown in Fig. 1, Oncomine analysis of neoplastic vs. normal tissue showed that the mRNA levels of *PDHA1* were significantly underexpressed in different types of GC across three analyses. Underexpression of *PDHA1* was found in intestinal-type GC ($P = 0.009$), diffuse-type GC ($P = 0.036$), and mixed-type GC ($P = 0.025$). Then,

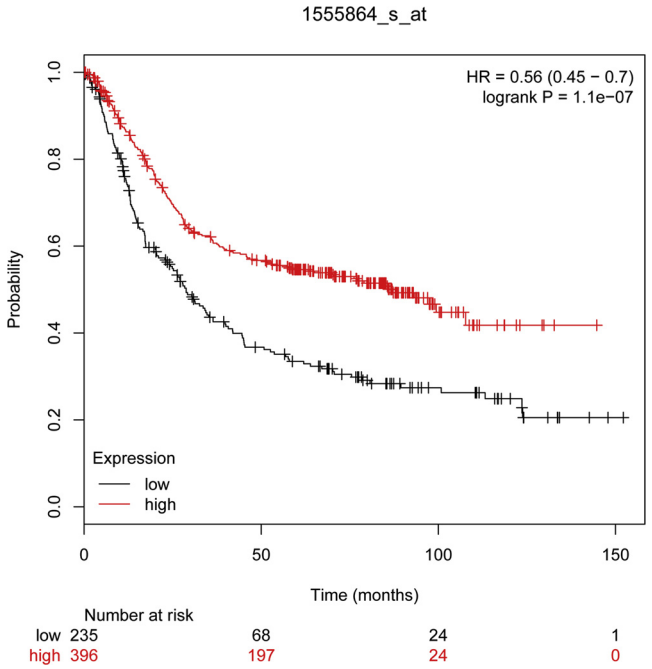

Fig. 2. *PDHA1* gene in GC (Kaplan-Meier Plotter). Kaplan-Meier plots showing overall survival in GC.

using the Kaplan-Meier Plotter online tool (http://kmplot.com/analysis/), we found that patients with GC expressing low levels of *PDHA1* had a poorer overall prognosis than those with high levels of *PDHA1* (P = 1.1e-07, Fig. 2).

3.2. PDHA1 immunohistochemical expression in TMA and association with clinicopathologic features

As shown in Fig. 3, immunohistochemical staining of the TMA showed that PDHA1 was predominantly localized in the cytoplasm. PDHA1 staining is much stronger in normal mucosa (IRS, 4.83 ± 0.48) than in GC (IRS, 3.69 ± 0.25) samples (P = 0.040). Furthermore, PDHA1 expression levels were found to be significantly lower in 69.05% (87/126) of poorly differentiated GCs as compared to the well or moderately differentiated ones (P = 0.037; Table 1), suggesting that the decreased expression of PDHA1 may be associated with GC progression. Intriguingly, PDHA1 expression was significantly correlated with depth of invasion (P < 0.001), lymph node metastasis (P < 0.001), TNM stage (P < 0.001), and nerve invasion (P = 0.006). However, it was not correlated with gender, age, Lauren classification, and lymphovascular invasion (P > 0.05 for all, Table 1).

3.3. Low PDHA1 protein expression correlates with a shorter overall survival

The Kaplan-Meier survival curves for patients in the different categories of PDHA1 protein expression are shown in Fig. 4. Low tumor expression of PDHA1 was significantly correlated with a poorer overall survival in patients with GC (5-year overall survival rates for patients with low vs high PDHA1 expression = 49.8% vs 72.7%, hazard ratio [HR] of death from GC = 2.594, 95% CI = 1.527 to 4.408, P < 0.001). In univariate analysis, gender, age, depth of invasion, Lauren classification, differentiation, lymphovascular invasion, and nerve invasion were not significantly associated with survival. Lymph node

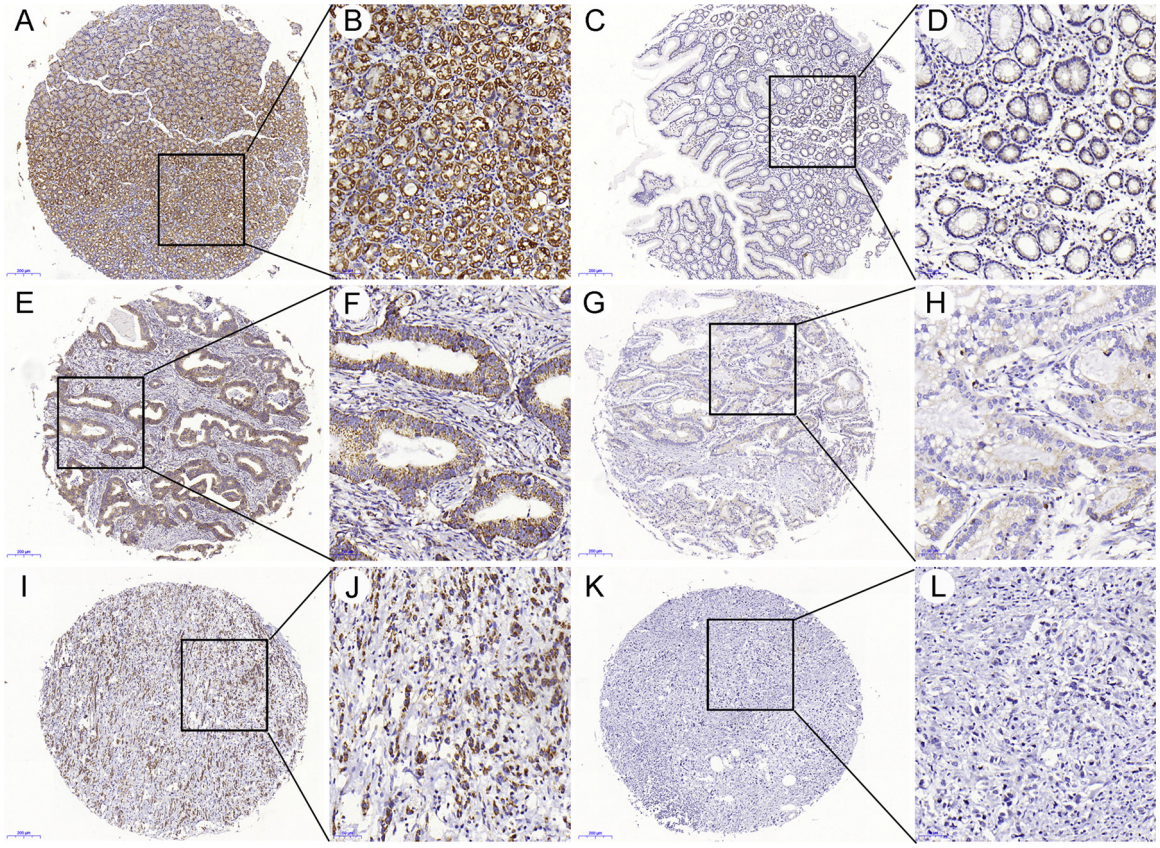

Fig. 3. Representative images of immunohistochemical staining of TMAs with anti-PDHA1 antibody. A and B, Normal mucosa with strong staining. C and D, Normal mucosa with weak staining. E and F, Well-differentiated GC with strong staining. G and H, Well-differentiated GC with weak staining. I and J, Poorly-differentiated GC with strong staining. K and L, Poorly-differentiated GC with negative staining (A, C, E, G, I, K: 40× magnification; B, D, F, H, J, L: 200× magnification).

Table 1
Relationship between expression levels of PDHA1 and clinicopathological features of 174 GC specimens.

Variables	n	PDHA1		χ^2	*P*
		Low	High		
Gender				0.228	0.633
Female	55	34	21		
Male	119	78	41		
Age (years)				0.441	0.507
≤65	104	69	35		
> 65	70	43	27		
Depth of invasion				16.061	*< 0.001*
T1/T2	38	14	24		
T3/T4	136	98	38		
Lymph node metastasis				14.978	*< 0.001*
N0	60	27	33		
N1/N2/N3	114	85	29		
TNM stage				17.883	*< 0.001*
I-II	86	42	44		
III-IV	88	70	18		
Lauren classification				4.625	0.990
Diffuse	92	66	26		
Intestinal	32	18	14		
Mixed	50	28	22		
Differentiation				4.361	*0.037*
Well/moderate differentiation	48	25	23		
Poor differentiation	126	87	39		
Lymphovascular invasion				3.692	0.055
Absent	125	75	50		
Present	49	37	12		
Nerve invasion				7.628	*0.006*
Absent	127	74	53		
Present	47	38	9		

Statistical analyses were performed by the Pearson χ^2 test.
A *P* value < 0.05 was considered significant. Italic indicates significant *P* values.

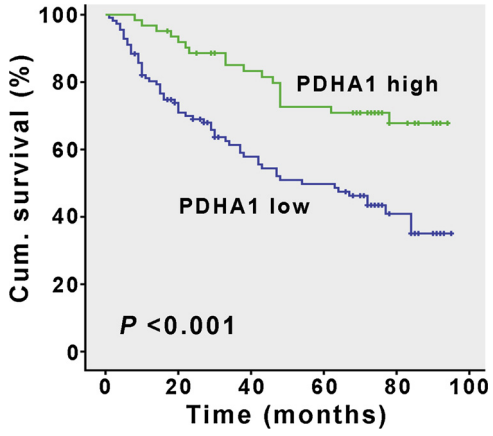

Fig. 4. Kaplan-Meier survival curves for 174 GC patients according to PDHA1 expression status (log-rank test, $P < 0.001$).

metastasis ($P < 0.001$), TNM stage ($P < 0.001$), and PDHA1 ($P < 0.001$) showed a statistically significant impact on survival (Table 2). Multivariate analysis showed that TNM stage ($P < 0.001$) and PDHA1 ($P = 0.025$) were independent predictors of overall survival (Table 2).

4. Discussion

GC is often diagnosed late, since symptoms may not appear until it is in the advanced stages [17]. Metabolic reprogramming is considered a hallmark of cancer. PDH, a mitochondrial multienzyme complex, plays a crucial role in energy metabolism. It catalyzes the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA [18]. PDH

Table 2
Univariate and multivariate analysis of prognostic factors in 174 GC patients.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Gender				
Female	1.00			
Male	0.957 (0.594 to 1.543)	0.857		
Age				
≤65 years	1.00			
> 65 years	1.562 (0.998 to 2.446)	0.051		
Depth of invasion				
T1/T2	1.00			
T3/T4	1.368 (0.787 to 2.375)	0.266		
Lymph node metastasis				
N0	1.00			
N1/N2/N3	3.423 (1.913 to 6.124)	*< 0.001*		
TNM stage				
I-II	1.00		1.00	
III-IV	3.381 (2.076 to 5.507)	*< 0.001*	2.824 (1.700 to 4.693)	*< 0.001*
Lauren classification				
Intestinal	1.00			
Diffuse	1.419 (0.734 to 2.744)	0.298		
Differentiation				
Well/moderate differentiation	1.00			
Poor differentiation	1.538 (0.887 to 2.668)	0.125		
Lymphovascular invasion				
Absent	1.00			
Present	1.433 (0.894 to 2.299)	0.135		
Nerve invasion				
Absent	1.00			
Present	1.099 (0.659 to 1.830)	0.718		
PDHA1				
High	1.00		1.00	
Low	2.594 (1.527 to 4.408)	*< 0.001*	1.880 (1.083 to 3.265)	*0.025*

Statistical analyses were performed by Cox proportional hazards regression.
A *P* value < 0.05 was considered significant. Italic indicates significant *P* values.
CI, confidence interval.

complex is comprised of multiple catalytic enzymes, including E1α (encoded by *PDHA1*), E1β, E2, and E3 subunits. Pyruvate decarboxylation catalyzed by PDHA1 is recognized as the rate-limiting step. E1 is activated by pyruvate dehydrogenase phosphatases and inactivated by pyruvate dehydrogenase kinases [19,20].

Recently, high PDHA1 expression is found to be correlated with better tumor differentiation in esophageal squamous cell carcinoma [12]. Intriguingly, PDHA1 is also found to be correlated with tumor differentiation, lymph node metastasis, and TNM stage in non-small cell lung cancer [21]. Moreover, low PDHA1 expression is significantly associated with higher FIGO stage in ovarian carcinoma [22]. Zhong et al. [14] report that PDHA1 is associated with the metabolic reprogramming and the cell stemness in prostate cancer cells. Sun et al. [15] report that upregulation of PDHA1 can inhibit Warburg effect and enhance mitochondria-mediated apoptosis pathway in hepatocellular carcinoma. Recently, Liu et al. [16] report that PDHA1 is downregulated in GC tissues, particularly in high-grade tumors. Upregulation of miR-21-5p expression inhibits PDHA1 expression by directly targeting PDHA1, thereby promoting glycolysis and cancer progression in GC. In the current study, Oncomine analysis of neoplastic vs. normal tissue showed that the mRNA levels of *PDHA1* were significantly

underexpressed in different types of GC across three analyses. Underexpression of *PDHA1* was found in intestinal-type GC ($P = 0.009$), diffuse-type GC ($P = 0.036$), and mixed-type GC ($P = 0.025$). Using TMAs, we demonstrated that PDHA1 was significantly lower in poorly differentiated GCs as compared to the well or moderately differentiated ones ($P = 0.037$). Furthermore, PDHA1 expression was significantly correlated with depth of invasion ($P < 0.001$), lymph node metastasis ($P < 0.001$), TNM stage ($P < 0.001$), and nerve invasion ($P = 0.006$). However, it was not correlated with gender, age, Lauren classification, and lymphovascular invasion ($P > 0.05$ for all). Previous studies report that decreased PDHA1 expression is associated with poor overall survival in patients with esophageal squamous cell carcinoma and prostate cancer [12,14]. Furthermore, low PDHA1 expression is also found to be significantly associated with shorter progression-free survival and overall survival in ovarian carcinoma [22]. Similarly, we found that low tumor expression of PDHA1 was significantly correlated with a poorer overall survival in patients with GC. Multivariate analysis showed that PDHA1 ($P = 0.025$) was an independent predictor of overall survival.

There were several limitations in our study. One major limitation is its retrospective character. A prospective study is needed to confirm our findings. Another limitation is that we utilize the TMAs to investigate PDHA1 expression, patterns of expression in TMAs might not represent the expression patterns in whole tissues. More importantly, additional in vitro studies are needed to elucidate the molecular mechanisms underlying the involvement of PDHA1 in the development and progression of GCs.

Taking these results together, our study showed that the decreased expression of PDHA1 may be associated with GC progression. Low PDHA1 staining was significantly correlated with a poorer overall survival in patients with GC, and PDHA1 was an independent predictor of overall survival. These findings are of potential clinical utility and merit further validation.

Conflict of interest

No potential conflicts of interest were disclosed.

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