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Original Article

Novel prognostic value of nuclear lactate dehydrogenase-A in esophageal squamous cell carcinoma

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Abstract: Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant tumors, and its prognosis is very poor. As a subunit of lactate dehydrogenase, LDH-A favors the conversion of pyruvate to lactate and is up-regulated in many tumors. It has been reported that LDH-A can promote cell growth and migration. However, the nuclear and cytoplasmic expression of LDH-A and the correlations and significance of this expression profile in ESCC have yet to be determined. In this study, the location of LDH-A protein in ESCC tissue from the histologically proven ESCC patients undergoing curative resection was determined by immunohistochemical staining. The nuclear and cytoplasmic expression of LDH-A was analyzed. Kaplan-Meier plots and the Cox proportional hazards regression model were used to analyze the prognostic value of nuclear and cytoplasmic LDH-A expression. The results showed that LDH-A protein in ESCC tissue was detected in both the nucleus and cytoplasm. High nuclear LDH-A expression was associated with malignant behavior, including tumor stage ($P = 0.026$) and lymph node metastasis ($P = 0.022$). High nuclear or cytoplasmic LDH-A expression alone was associated with lower overall survival (OS) rates (8.1%, $P = 0.004$ and 13.2%, $P = 0.038$, respectively). Moreover, in multivariate analysis, high nuclear LDH-A expression was the most significant factor indicating poor prognosis in ESCC patients (95% CI: 1.215~3.658, $P = 0.008$). In conclusion, our study showed that nuclear and cytoplasmic LDH-A expression correlate with more aggressive tumor behavior in ESCC and that nuclear LDH-A expression may serve as an effective prognostic marker in patients with surgically resected ESCC.

Keywords: Lactate dehydrogenase A, nuclear expression, esophageal squamous cell carcinoma, immunohistochemistry, prognosis

Introduction

Worldwide, the incidence and mortality of the two main types of esophageal carcinoma, squamous cell carcinoma and adenocarcinoma, remain high [1]. As the dominant type of esophageal cancer in China, the incidence of esophageal squamous cell carcinoma (ESCC) ranks sixth, while mortality due to ESCC ranks fourth. Moreover, these incidence and mortality rates have increased yearly from 2007 and 2008, respectively. Because the symptoms are not obvious at an early stage, ESCC patients are commonly diagnosed at an advanced stage; moreover, the lack of effective clinical methods for early detection and promising therapies together result in a poor prognosis [2, 3]. Therefore, prognostic biomarkers are needed to identify the subset of patients with a high risk of disease failure to guide individualized treatment.

Lactate dehydrogenase (LDH) is a 140-kDa tetrameric molecule composed of two different subunits: LDH-A and LDH-B. LDH-A is mainly located in the cytoplasm, catalyzing the posi-

Table 1. Scoring criteria of nuclear LDHA expression

Expression intensity	Expression range			
	0%	0~10%	10~20%	≥ 20%
-	Negative	/	/	/
+	/	Weak	Weak	Moderate
++	/	Weak	Moderate	Strong
+++	/	Moderate	Strong	Strong

Table 2. Scoring criteria of cytoplasmic LDH-A expression

Expression intensity	Expression range			
	0%	0~35%	35~70%	≥ 70%
-	Negative	/	/	/
+	/	Weak	Weak	Moderate
++	/	Weak	Moderate	Strong
+++	/	Moderate	Strong	Strong

tive reaction in the last step of the glycolytic pathway [4, 5]. Unlike normal cells, cancer cells generate the ATP for cancer cell growth and division through glycolysis [6], and the abnormal expression of key enzymes in the glycolytic pathway may contribute to the growth of cancer cells. For instance, previous studies have found that PKM2 (pyruvate kinase M2), one of the key enzymes in the glycolytic pathway, significantly predicts the prognosis of ESCC patients [7, 8]. The abnormal expression of LDH-A has also been documented in many tumors [9-11].

LDH-A has been reported to be involved in tumor progression [12-14], and elevated levels of LDH-A, which are found in various human cancers, are correlated with tumor size and poor prognosis [9-11]. Rong et al. demonstrated that LDH-A promoted cell growth in pancreatic cancer [9], and Yao et al. showed that *LDH-A* knockdown could inhibit cell growth and migration of ESCC in vitro and tumorigenesis in vivo [13]. In addition to the expression of LDH-A in the cytoplasm, where it mediates cell energy metabolism, this enzyme is also present in mitochondria and the nucleus, although its precise role in the nucleus has yet to be fully elucidated [15]. Because LDH-A is distributed within transcriptionally active regions of chromosomes, it may be involved in regulating the transcription of other genes and participate in regulating survival pathways [5], thereby affecting the malignant behavior of tumors and the treatment outcome of cancer patients.

The purpose of this study was to evaluate the nuclear and cytoplasmic expression of LDH-A and to analyze the correlation between this expression pattern and the clinicopathological parameters of ESCC. Moreover, the influence of nuclear and cytoplasmic LDH-A expression on the overall survival (OS) of patients with ESCC was assessed.

Materials and methods

Patient samples and characteristics

Eighty-four patients with diagnosed and pathologically confirmed ESCC were evaluated in the Meizhou People's Hospital of Guangdong Province. Demographic and clinicopathological data, including age, sex, histological grade, tumor size, depth of invasion, and clinical stage (TNM stage), were collected from patient medical records. All patients underwent curative surgery between June 2003 and June 2007, and none of them received radiotherapy or chemotherapy prior to curative surgery. Written informed consent was obtained from all ESCC patients, and this study was approved by the research Ethics Committee of Meizhou People's Hospital.

Immunohistochemical staining

Slides (4 μm) from formalin-fixed, paraffin-embedded tissue samples were used for immunohistochemistry (IHC). Tissue sections were dried in an oven at 65°C for 60 min. After deparaffinization and hydration in buffer (water), epitope retrieval was performed by boiling the sections in citrate buffer solution (pH = 6.0) for 10 min. The slides were then placed in buffer for at least 20 minutes at room temperature and washed three times for 3 min each in PBS. The sections were then incubated for 2~3 h at room temperature with rabbit anti-human LDH-A antibody (1:400; CST) or PBS as a negative control. After three washes in PBS, the slides were incubated with goat anti-rabbit/mouse horseradish peroxidase polymer (Envision PO System; DAKO) for 30 min followed by incubation with 3,3'-diaminobenzidine as chromogen for 30~60 s. The sections were counterstained for 6 min with Mayer's hematoxylin and permanently mounted. The staining pattern (cytoplas-

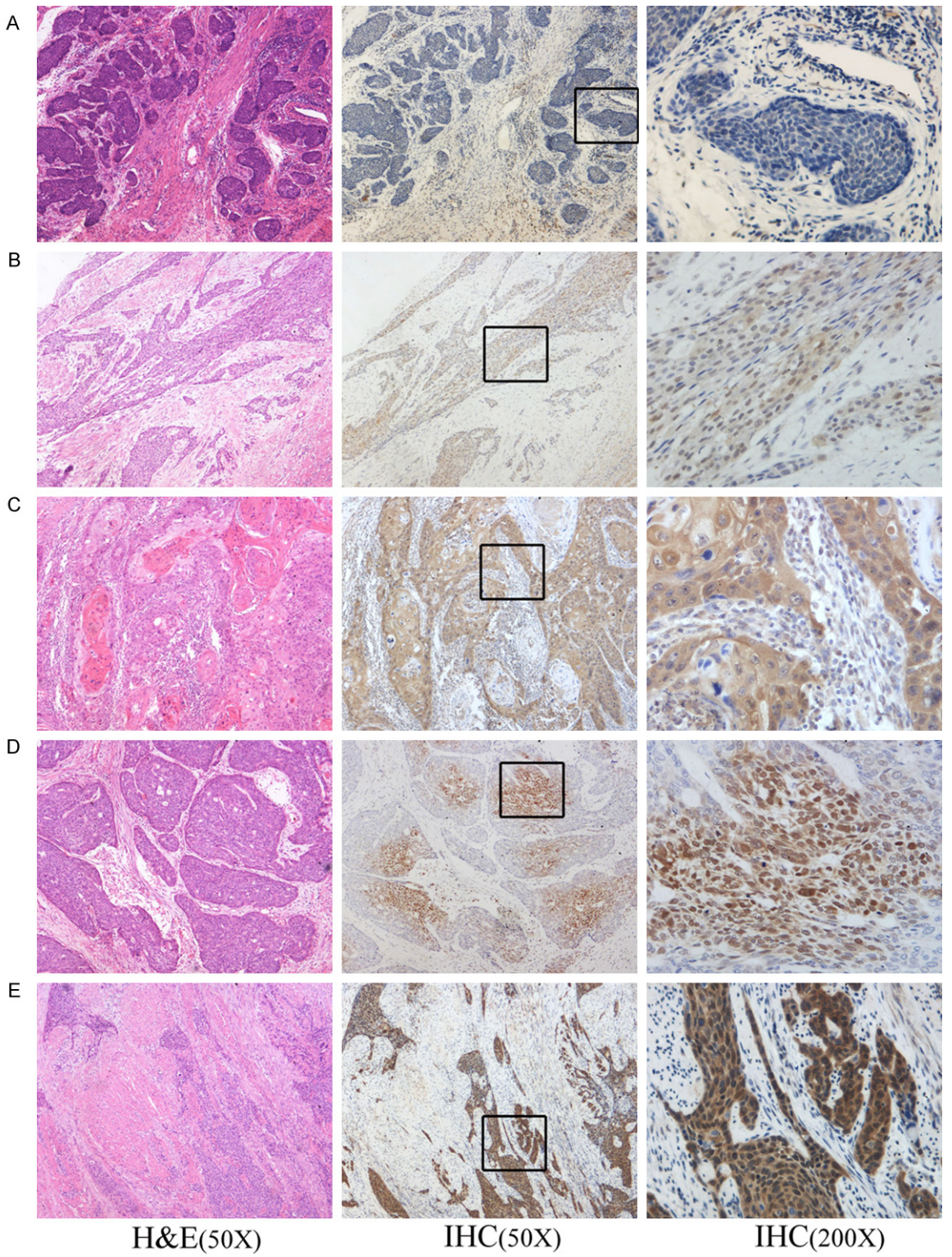

Figure 1. Expression of nuclear and cytoplasmic LDH-A by immunohistochemical staining, showing five typical ESCC staining patterns. Representative ESCC tumor samples showing the expression of nuclear and cytoplasmic LDH-A. A. Dual negative expression of nuclear and cytoplasmic LDH-A. B. Dual low expression of nuclear and cytoplasmic LDH-A. C. High cytoplasmic and low nuclear expression of LDH-A. D. Low cytoplasmic and high nuclear expression of LDH-A. E. Dual high expression of nuclear and cytoplasmic LDH-A. Left column: H&E staining, 50×; middle column: IHC, 50×; right column: black box area from the middle column by IHC, 200×.

Table 3. Correlation between clinicopathological parameters and nuclear or cytoplasmic LDH-A expression

Variable	N	$LDHA^{Nuc*}$ expression		*P* value	$LDHA^{Cyto**}$ expression		*P* value
		Low	High		Low	High	
Gender				**0.005**			**0.025**
Male	68	33 (48.5%)	35 (51.5%)		22 (32.4%)	46 (67.6%)	
Female	16	14 (87.5%)	2 (12.5%)		10 (62.5%)	6 (37.5%)	
Age (years)				0.196			**0.012**
≤ 55	43	27 (62.8%)	16 (37.2%)		22 (51.2%)	21 (48.8%)	
> 55	41	20 (48.8%)	21 (51.2%)		10 (24.4%)	31 (75.6%)	
Histological grade				0.222			0.309
I	13	10 (76.9%)	3 (23.1%)		3 (23.1%)	10 (76.9%)	
II	64	34 (53.1%)	30 (46.9%)		25 (39.1%)	39 (60.9%)	
III	7	3 (42.9%)	4 (57.1%)		4 (57.1%)	3 (42.9%)	
Tumor size				0.867			0.578
≤ 5 cm	44	25 (56.8%)	19 (43.2%)		18 (40.9%)	26 (59.1%)	
> 5 cm	40	22 (55.0%)	18 (45.0%)		14 (35.0%)	26 (65.0%)	
Depth of invasion				0.490			0.595
Muscular	26	16 (61.5%)	10 (38.5%)		11 (42.3%)	15 (57.7%)	
Serosa	58	31 (53.4%)	27 (46.6%)		21 (36.2%)	37 (63.8%)	
Tumor stage				**0.026**			0.140
I+II	52	34 (65.4%)	18 (34.6%)		23 (44.2%)	29 (55.8%)	
III+IV	32	13 (40.6%)	19 (59.4%)		9 (28.1%)	23 (71.9%)	
Lymph nodes metastases				**0.022**			0.436
Negative	48	32 (66.7%)	16 (33.3%)		20 (41.7%)	28 (58.3%)	
Positive	36	15 (41.7%)	21 (58.3%)		12 (33.3%)	24 (66.7%)	

Bold values indicate statistically significant differences. *Nuclear LDH-A; **Cytoplasmic LDH-A.

mic or nuclear) was recorded, and the expression was evaluated separately for the nucleus and cytoplasm.

Statistical analysis

The detailed procedure for LDH-A staining is shown in **Tables 1** and **2**. Negative and weak expressions were classified as low expression, while moderate and strong expression was classified as high expression. The data were analyzed with SPSS software version 19.0. The Chi-square test was used to analyze the association between $LDH\text{-}A^{nuc/cyto}$ expression and clinicopathological features. Kaplan-Meier survival curves were employed, and the statistics were evaluated using the log-rank method to compare overall survival rates, while Cox regression analysis was used for the multivariate analysis. For all statistical analyses, only *P* values < 0.05 were considered statistically significant, and all *P* values were two-sided.

Results

Clinical characteristics and expression of LDH-A in 84 ESCC patients

Our study included 68 males and 16 females, and the gender ratio of male to female was 4.25:1. The median age was 55 years (range 41-75 years). The numbers of grade I, II and III ESCC cases were 13 (15.5%), 64 (76.2%) and 7 (8.3%), respectively. The median tumor size (maximum diameter) was 5 cm, and the cases were classified into two groups based on depth of invasion: muscular layer, 26 cases, 31.0%; and serosa layer, 58 cases, 69.0%. Tumor stage was distributed among cases as follows: I+II, 52 cases (61.9%); III+IV, 32 cases (38.1%).

LDH-A protein was detected in the nucleus and cytoplasm, as shown in **Figure 1**. The number of cases with low and high nuclear expression of LDH-A was 47 (56.0%) and 37 (44.0%), respectively, and the number of cases with low

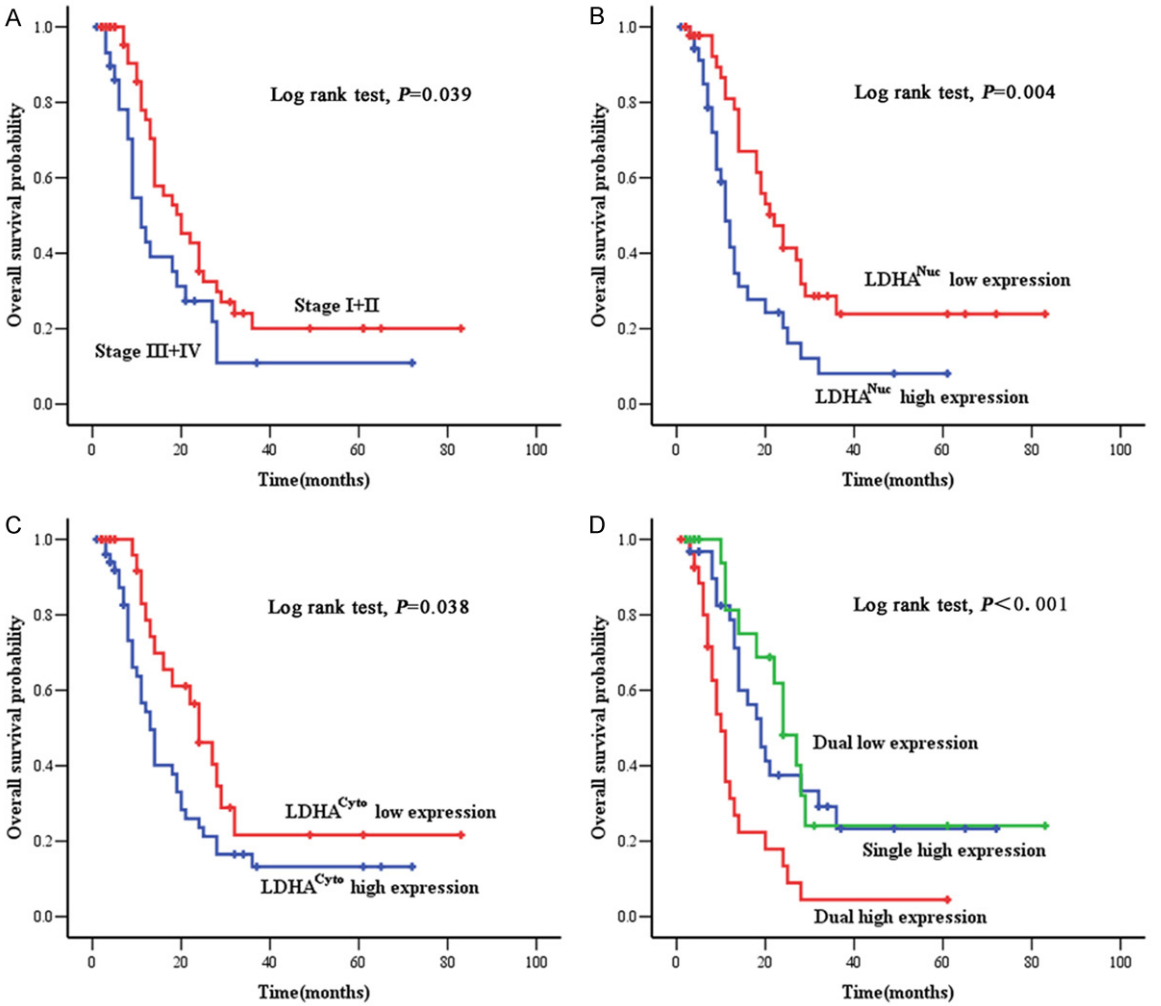

Figure 2. Relationship between tumor stage, nuclear and cytoplasmic LDH-A expression and OS in 84 ESCC patients. A. Patients with an advanced tumor stage had a poorer prognosis (P = 0.039); B, C. High nuclear and cytoplasmic LDH-A expression were significantly correlated with poorer prognosis (P = 0.004, 0.038); D. Dual high nuclear and cytoplasmic LDH-A expression was significantly correlated with poor prognosis (P < 0.001).

and high cytoplasmic expression of LDH-A was 32 (38.1%) and 52 (61.9%), respectively.

Relationship between nuclear/cytoplasmic LDH-A expression and ESCC patients' clinicopathological variables

Associations between nuclear/cytoplasmic expression in ESCC and clinicopathological variables were assessed, as displayed in **Table 3**. High expression of nuclear LDH-A was found to significantly correlate with male gender (P = 0.005), advanced tumor stage (P = 0.026) and more lymph nodes metastases (P = 0.022). In addition, high expression of cytoplasmic LDH-A was significantly correlated with male gender (P = 0.025) and older age (P = 0.012). No significant correlation was detected between nuclear/cytoplasmic LDH-A expression level and other clinicopathological variables (P > 0.05).

Relationships between clinicopathological variables, nuclear and cytoplasmic LDH-A expression, and overall survival by univariate analysis

The follow-up information of the 84 patients was collected within the range from 1 to 83 months after surgery. The mean and median OS time was 27.08 months and 16.00 months, respectively, and the 3-year OS rate was 16.9%.

Univariate analysis showed that the OS rate in patients with high expression of nuclear/cytoplasmic LDH-A was significantly lower than that in patients with low expression (**Figure 2**). As shown in **Table 4**, the mean/median survival

Table 4. Univaraite survival analysis of 84 ESCC patients by log-rank test

Patients features	N	Mean ± SE (months)	Median ± SE (months)	3-year OS rate (%)	*P* value
Gender					0.239
Male	68	25.70 ± 3.69	14.00 ± 2.40	15.7	
Female	16	29.33 ± 5.61	24.00 ± 4.27	25.0	
Age at surgery (years)					0.843
≤ 55	43	26.55 ± 5.04	14.00 ± 2.15	16.9	
> 55	41	25.53 ± 3.89	20.00 ± 3.46	16.7	
Tumor size (cm)					0.832
≤ 5	44	23.31 ± 2.91	19.00 ± 2.21	13.4	
> 5	40	28.29 ± 5.55	13.00 ± 1.34	21.3	
Depth of invasion					0.431
Muscular layer	26	28.95 ± 6.19	19.00 ± 7.94	16.1	
Serosa layer	58	24.52 ± 3.42	14.00 ± 2.40	17.4	
Tumor stage					**0.039**
I+II	52	30.73 ± 4.47	20.00 ± 3.76	20.1	
III+IV	32	19.54 ± 4.18	11.00 ± 1.43	10.9	
$LDHA^{Nuc}$ expression					**0.004**
Low expression	47	33.97 ± 5.00	22.00 ± 2.89	23.9	
High expression	37	16.86 ± 2.82	11.00 ± 1.05	8.1	
$LDHA^{Cyto}$ expression					**0.038**
Low expression	32	33.55 ± 6.23	24.00 ± 3.35	21.6	
High expression	52	21.48 ± 3.28	13.00 ± 1.39	13.2	
$LDHA^{Nuc}$ and $LDHA^{Cyto}$ expression					**0.000**
Dual low expression	23	35.52 ± 7.37	24.00 ± 2.95	24.1	
Single high expression	33	29.93 ± 4.86	19.00 ± 2.56	23.3	0.977*
Dual high expression	28	13.82 ± 2.55	10.00 ± 1.16	4.5	

Bold values indicate statistically significant differences. **P* value of overall survival between patients with high nuclear and low cytoplasmic LDH-A expression and low nuclear and high cytoplasmic LDH-A expression.

Table 5. Multivaraite survival analysis of 84 ESCC patients by Cox regression

Variable	Hazard ratio (HR)	95% confidence interval of HR	*P* value
Tumor stage*	1.737	1.001~3.017	0.050
$LDHA^{Nuc}$ expression**	2.108	1.215~3.658	0.008
$LDHA^{Cyto}$ expression**	1.683	0.933~3.035	0.084

*Stages III+IV versus stages I+II; **High expression versus low expression.

time was significantly shorter for patients with high nuclear LDH-A expression than for those with low nuclear LDH-A expression (16.86 months vs. 33.97 months, 11 months vs. 22 months; $P = 0.004$). The 3-year OS rates for these groups were 8.1% and 23.9%, respectively. Patients with high cytoplasmic LDH-A expression also showed a shorter mean/median survival time than those with low cytoplasmic LDH-A expression (21.48 months vs. 33.55 months, 13 months vs. 24 months; $P = 0.038$). The 3-year OS rates for these groups were 13.2% and 21.6%, respectively.

Interestingly, when combining nuclear and cytoplasmic LDH-A expression, the OS rate of patients with dual high expression of nuclear and cytoplasmic LDH-A was substantially lower than that of patients with single high or dual low nuclear and cytoplasmic LDH-A ($P < 0.001$). Moreover, tumor stage also had a significant adverse impact on OS ($P = 0.039$).

Prognostic significance of nuclear and cytoplasmic LDH-A expression in ESCC in multivariate analysis

As shown in **Table 5**, multivariate analysis revealed that high expression of nuclear LDH-A

remained as the only independent prognostic factor for OS (hazard ratio (HR): 2.108, 95% confidence interval (CI): 1.215~3.658, P = 0.008) compared with cytoplasmic LDH-A (HR: 1.683, 95% CI: 0.933~3.035, P = 0.084) and tumor stage (HR: 1.737, 95% CI: 1.001~3.017, P = 0.050).

Discussion

ESCC is the most common subtype of esophageal carcinoma, with an increasing incidence and high mortality in China [2]. Following continuous improvements in medicine in recent decades, diagnostic technologies and therapeutic strategies have greatly improved; however, the prognosis of ESCC remains poor, with a 5-year OS rate of approximately 10-41% [16, 17]. Therefore, it is crucial to identify a novel biomarker to predict the prognosis of patients and guide individualized treatment.

Malignant tumors are not only considered a genetic disease but also a metabolic disease, as alterations in glycolysis plays an important role in tumorigenesis [18]. LDH-A expression is mainly detected in the cytoplasm, and this enzyme catalyzes the positive reaction of the final step of glycolysis, converting pyruvate to lactate. Yao et al. showed that LDH-A promoted cell growth in the ESCC cell lines Eca109 and Caes17 via regulating the expression of CyclinD1, cleaved PARP and caspase 8, and knockdown of the expression of LDH-A was shown to inhibit tumorigenesis in vivo [13]. Indeed, inhibition of LDH-A expression suppresses tumor growth and metastasis and even causes the death of tumor cells [9, 19]. In this study, we found that 61.9% of ESCC patients had high expression of cytoplasmic LDH-A, which was correlated with poor survival. Many reports have demonstrated that total LDH-A is a potential prognostic marker in many tumors [10, 11], and our study also found that ESCC patients with high cytoplasmic LDH-A expression had a shorter mean/median survival time than those with low cytoplasmic LDH-A expression. In addition, high expression of cytoplasmic LDH-A was found to significantly correlate with male gender (P = 0.025) and older age (P = 0.012). In a similar finding, the expression of PKM2 was found to be much higher in ESCC than in normal tissue, and this expression is highly associated with many adverse clinical features and prognoses in ESCC, as reported by Zhan C et al. [7].

In addition to the cytoplasm, LDH-A has also been detected in mitochondria and nuclei. However, the role of nuclear LDH-A is still far from clear. The current study found that the expression level of LDH-A in the nucleus was high in 37 (44%) patients. Furthermore, significant correlations were detected between high expression of nuclear LDH-A and clinicopathological variables including advanced tumor stage (P = 0.026) and positive lymph nodes metastasis (P = 0.022), suggesting that LDH-A may play an important role in ESCC development. Nuclear LDH was found to localize to transcriptionally active regions of chromosomes [5, 20], and other studies have described nuclear LDH-A as mainly a single-stranded DNA-binding protein [21]. Together, these findings imply that nuclear LDH-A may have a similar function as other ssDNA-binding proteins, such as possible involvement in DNA transcription and/or replication and regulating the transcription of other genes, thereby regulating genes and proteins participating in tumor progression. Recently, Castonguay et al. reported that nuclear LDH can promote histone deacetylation by modulating the availability of NAD^+, an essential ingredient for the activity of Sirtuin type 1 (SIRT1), which is intimately related to cell proliferation, differentiation, apoptosis, and metabolism [15]. Nuclear LDH-A may play a role in tumor progression by affecting histone deacetylation. Thus, it is not surprising that nuclear LDH-A expression showed good predictive value for the prognosis of ESCC patients in our univariate analysis.

High expression of LDH-A in the cytoplasm and nucleus appears to provide a metabolic and growth advantage to ESCC cells. Accordingly, patients with dual high expression of nuclear and cytoplasmic LDH-A showed the worst OS (P < 0.001). Moreover, multivariate analysis showed that nuclear LDH-A expression had the greatest potential value in prognostic evaluation (HR = 2.108, P = 0.008). Many studies have shown that total LDH-A overexpression and/or increased plasma LDH are important factors affecting the prognosis of cancer patients [22, 23], but few reports have focused on nuclear LDH-A expression. Our study is the first to examine both the nuclear and cytoplasmic expression of LDH-A in ESCC, and our results suggest that nuclear LDH-A may be even more important when evaluating ESCC progression. The subcellular localization of

LDH-A should be taken into account in risk estimation and treatment consideration for ESCC patients, and the underlying mechanism warrants further investigation.

Interestingly, we also found that LDH-A expression was higher in male than female patients in both the nucleus and cytoplasm (P = 0.005; 0.025). The association of LDH-A expression with gender may be attributable to hormone levels, which requires further study.

Taken together, the results of this study suggest that the expression of nuclear LDH-A may serve as a prognostic indicator for poor survival in ESCC patients.

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Disclosure of conflict of interest

None.

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References

[1] Rustgi AK and El-Serag HB. Esophageal carcinoma. N Engl J Med 2014; 371: 2499-2509.

[2] Tang WR, Fang JY, Wu KS, Shi XJ, Luo JY and Lin K. Epidemiological characteristics and prediction of esophageal cancer mortality in China from 1991 to 2012. Asian Pac J Cancer Prev 2014; 15: 6929-6934.

[3] Chen JW, Xie JD, Ling YH, Li P, Yan SM, Xi SY, Luo RZ, Yun JP, Xie D and Cai MY. The prognostic effect of perineural invasion in esophageal squamous cell carcinoma. BMC Cancer 2014; 14: 313.

[4] Doherty JR and Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest 2013; 123: 3685-3692.

[5] Fiume L, Manerba M, Vettraino M and Di Stefano G. Inhibition of lactate dehydrogenase activity as an approach to cancer therapy. Future Med Chem 2014; 6: 429-445.

[6] Vander Heiden MG, Cantley LC and Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-1033.

[7] Zhan C, Shi Y, Lu C and Wang Q. Pyruvate kinase M2 is highly correlated with the differentiation and the prognosis of esophageal squamous cell cancer. Dis Esophagus 2013; 26: 746-753.

[8] Zhang X, He C, He C, Chen B, Liu Y, Kong M, Wang C, Lin L, Dong Y and Sheng H. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. Pathol Res Pract 2013; 209: 510-515.

[9] Rong Y, Wu W, Ni X, Kuang T, Jin D, Wang D and Lou W. Lactate dehydrogenase A is overexpressed in pancreatic cancer and promotes the growth of pancreatic cancer cells. Tumour Biol 2013; 34: 1523-1530.

[10] Girgis H, Masui O, White NM, Scorilas A, Rotondo F, Seivwright A, Gabril M, Filter ER, Girgis AH, Bjarnason GA, Jewett MA, Evans A, Al-Haddad S, Siu KM and Yousef GM. Lactate dehydrogenase A is a potential prognostic marker in clear cell renal cell carcinoma. Mol Cancer 2014; 13: 101.

[11] Sun X, Sun Z, Zhu Z, Guan H, Zhang J, Zhang Y, Xu H and Sun M. Clinicopathological significance and prognostic value of lactate dehydrogenase A expression in gastric cancer patients. PLoS One 2014; 9: e91068.

[12] Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL and Dang CV. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci U S A 2010; 107: 2037-2042.

[13] Yao F, Zhao T, Zhong C, Zhu J and Zhao H. LDHA is necessary for the tumorigenicity of esophageal squamous cell carcinoma. Tumour Biol 2013; 34: 25-31.

[14] Miao P, Sheng S, Sun X, Liu J and Huang G. Lactate dehydrogenase A in cancer: a promising target for diagnosis and therapy. IUBMB Life 2013; 65: 904-910.

[15] Castonguay Z, Auger C, Thomas SC, Chahma M and Appanna VD. Nuclear lactate dehydrogenase modulates histone modification in human hepatocytes. Biochem Biophys Res Commun 2014; 454: 172-177.
[16] Baba Y, Watanabe M, Yoshida N and Baba H. Neoadjuvant treatment for esophageal squamous cell carcinoma. World J Gastrointest Oncol 2014; 6: 121-128.
[17] Yang Z, Wu Z, Liu T, Han L, Wang C, Yang B and Zheng F. Upregulation of PDK1 associates with poor prognosis in esophageal squamous cell carcinoma with facilitating tumorigenicity in vitro. Med Oncol 2014; 31: 337.
[18] Zhou W, Liotta LA and Petricoin EF. Cancer metabolism: what we can learn from proteomic analysis by mass spectrometry. Cancer Genomics Proteomics 2012; 9: 373-381.
[19] Sheng SL, Liu JJ, Dai YH, Sun XG, Xiong XP and Huang G. Knockdown of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma. FEBS J 2012; 279: 3898-3910.
[20] Williams KR, Reddigari S and Patel GL. Identification of a nucleic acid helix-destabilizing protein from rat liver as lactate dehydrogenase-5. Proc Natl Acad Sci U S A 1985; 82: 5260-5264.
[21] Augoff K, Hryniewicz-Jankowska A and Tabola R. Lactate dehydrogenase 5: An old friend and a new hope in the war on cancer. Cancer Lett 2015; 358: 1-7.
[22] Sun X, Sun Z, Zhu Z, Li C, Zhang J, Xu H and Sun M. Expression of SIP1 is strongly correlated with LDHA and shows a significantly poor outcome in gastric cancer. Tumour Biol 2015; 36: 7521-30.
[23] Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Trarbach T, Folprecht G, Shi MM, Lebwohl D, Jalava T, Laurent D, Meinhardt G and Harris AL. Prognostic and predictive role of lactate dehydrogenase 5 expression in colorectal cancer patients treated with PTK787/ZK 222584 (vatalanib) antiangiogenic therapy. Clin Cancer Res 2011; 17: 4892-4900.