Int J Clin Exp Med 2017;10(6):9771-9778
www.ijcem.com /ISSN:1940-5901/IJCEM0055505

Original Article

Studies on the PDHA1 protein expression and its correlation with clinicopathological characteristics and prognosis in NSCLC

Jing Cao[1,2*], Qiuge Wu[3*], Zhipai Lv[2], Xianxu Zeng[2], Qiuhong Dang[2], Zhenhe Suo[1,4,5]

Departments of [1]Oncology, [3]Respiratory and Critical Disease, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China; [2]Department of Pathology, The Third Affiliated Hospital of Zhenzhou University, Zhengzhou, Henan Province, China; [4]Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, University of Oslo, Montebello, Oslo 0379, Norway; [5]Department of Pathology, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo 0379, Norway. [*]Equal contributors and co-first authors.

Received April 15, 2017; Accepted May 9, 2017; Epub June 15, 2017; Published June 30, 2017

Abstract: Objective: To investigate the expression changes of PDHA1 in non-small cell lung cancer (NSCLC) and its correlation with clinicopathological characteristics and prognoses. Methods: The PDHA1 protein expressions in 196 NSCLC tissues were immunohistochemically detected. The χ^2 test was adopted to analyze the correlations between the expressions (positive/negative) and clinicopathological characteristics, such as patients' gender, pathological type, smoking history, degree of tumor differentiation, lymph node metastasis, TNM staging, etc. Besides, Kaplan-Meier survival analysis and multivariate factors regression analysis were performed to identify the relevant factors affecting the prognoses of patients with NSCLC. Results: The positive expression rate of PDHA1 was 38.8% (76/196) in lung cancer tissues and 96.0% (96/100) in adjacent normal tissues, the difference was statistically significant (P=0.018). No relationship was found between the PDHA1 protein expression and patients' gender, smoking history or pathological type, but it was found that the PDHA1 protein expression in patients with high and intermediate differentiation degree was higher than those with low differentiation (P=0.000). Patients without lymphatic metastasis showed higher PDHA1 protein expression than those with lymph node metastasis (P=0.015). Patients in stage I and II had higher PDHA1 expression than those in stage III and IV (P=0.037). According to the Cox's multivariate factors regression analysis, prognosis of NSCLC was related to the expression of PDHA1, the degree of tissue differentiation, lymph node metastasis and TNM staging (P < 0.001). Conclusion: The low expression of PDHA1 is relevant with the occurrence and development of NSCLC. Thus, PDHA1 could be a vital reference index to determine its biological behavior and prognosis.

Keywords: PDHA1, NSCLC, prognosis

Introduction

Primary lung cancer is one of malignant tumors with the highest morbidity and mortality in China as well as in the world; its morbidity and mortality have been increasing annually. And non-small cell lung cancer (NSCLC) accounts for over 80% of primary lung cancer [1]. The energy metabolism is the premise of cell survival. And the normal expression of PDHA1 protein is the premise of the normal tricarboxylic acid cycle and oxidative phosphorylation in mitochondrion. As a key enzyme, PDHA1 protein can connect the glycolysis with mitochondrial tricarboxylic acid cycle. Thus, the loss of PDHA1 can lead to the enzymatic inactivation of PDHc, and result in the inability of transferring pyruvic acid to acetyl-CoA, which can further trigger the block of the tricarboxylic acid cycle [2].

With the continuous development of the studies on tumor metabolism, scholars have started focusing on the impact of PDHA1 on tumor energy metabolism. The latest studies indicate that PDHA1 gene knockout in prostate cancer cell can up-regulate the expression level of glutaminase1 and glutamine dehydrogenase 1. Progressively, the metabolic pathway of tumor cells can be remodeled [3]. This study indicated

that PDHA1 could alter biological features of tumor by influencing its energy metabolism. Although several studies have figured out that pyruvate dehydrogenase (PDH) can severely obstruct the prognoses of patients with NSCLC by promoting the expression of hypoxia inducible factor-α [4]. However, the impact of the expression of PDHA1, a vital part of PDH, on patients with NSCLS is not clear yet. Therefore, 196 NSCLC tissues were immunohistochemically analyzed in this study in order to investigate the relationship between the expression level of PDHA1 protein and the clinicopathological characteristics as well as the prognoses.

Materials and methods

Clinical materials

A total of 196 cases, which were diagnosed as NSCLC from Jan. 2011 to Dec. 2013 in the First Affiliated Hospital of Zhengzhou University, were enrolled in this study. The diagnostic criterion met the Diagnosis and Treatment of Primary Lung Cancer in China (2015 Edition). All patients signed the informed consent and the study was approved by the Ethics Committee in our hospital. All the patients' clinical materials were abstracted from pathological archives, including operation record, tumor size, tumor staging, metastasis and postoperative adjuvant therapy. All the patients were followed up and 100 adjacent normal tissues (5 cm away from the tumor) were selected for comparison. All the lung cancer specimens, including operative specimens, bronchoscopic biopsy specimens and percutaneous lung puncture specimens guided by CT/B-mode ultrasonography, etc., were all preserved carefully.

Methods

Immunohistochemistry: Having fixed in 10% formaldehyde and embedded in paraffin wax, these tissues were sectioned serially and dewaxed. And antigen retrieval was performed by microwave heating. After being rinsed and closed, the sections were incubated in normal saline. Afterwards, the PDHA1 monoclonal antibody (bought from Thermo Fisher Scientific Co., diluted 1:100) was added and sections were incubated overnight at 4°C. Then the incubations were performed for 30 min at 37°C with the second antibody (bought form Thermo Fisher Scientific Co.) added. After that, working fluid marked by horseradish enzyme was dropped and the incubations were performed again for 30 min at 37°C. In the end, the sections were developed with DAB, counterstained with hematoxylin, dehydrated through graded alcohols and mounted. The above operative procedure was performed in strict accordance with the instruction on kits. After the completion of these operations, immunohistochemical staining sections were observed and imaged under a microscope, then analyzed by Image-ProPlus 6.0 to determine the experimental results.

Criteria of immunohistochemistry PDHA1 positive cell: The positive expression of PDHA1 protein was located in the cytoplasm. The dyeing intensity scoring criteria were as follows: 0 score, no staining, 1 score, thin yellow particles for a pile, 2 scores, thin dark-yellow particle diffuse, 3 scores, thick brown particles diffuse. The dyeing range rating criteria were as follows: 0 score, no positive cells, 1 score, ≤ 10% positive cells, 2 scores, 10% < x ≤ 50%, 3 scores, 50% < x ≤ 80%, 4 scores, > 80%. Then the product of multiplication of two scores were used for dividing the sample into the negative group (0-4 scores) and the positive group (5-12 scores).

Follow-up observation: After the surgery, patients were followed up every 3 months in the first two years and every 6 months thereafter. The deadline of follow-up was in March, 2016, and the loss to follow up and the death during this period were defined as censored data. The follow-up mainly contained the examination of patients' physical condition, recurrence and metastasis of tumor and the patients' living condition, with the methods of physical examination, chest X-ray plain film, CT, fiberoptic bronchoscope examination, whole body bone scan, PET-CT and so forth. All patients' relevant test results were recorded and the overall survival (OS) was calculated. OS was calculated from the date of diagnosis of lung cancer to the date of death or last follow-up date with a time horizon of months. Follow-up information of most cases was obtained.

Statistical analysis

Enumeration data analyses were examined by Pearson χ^2, and survival analyses were performed by using Kaplan-Meier method and

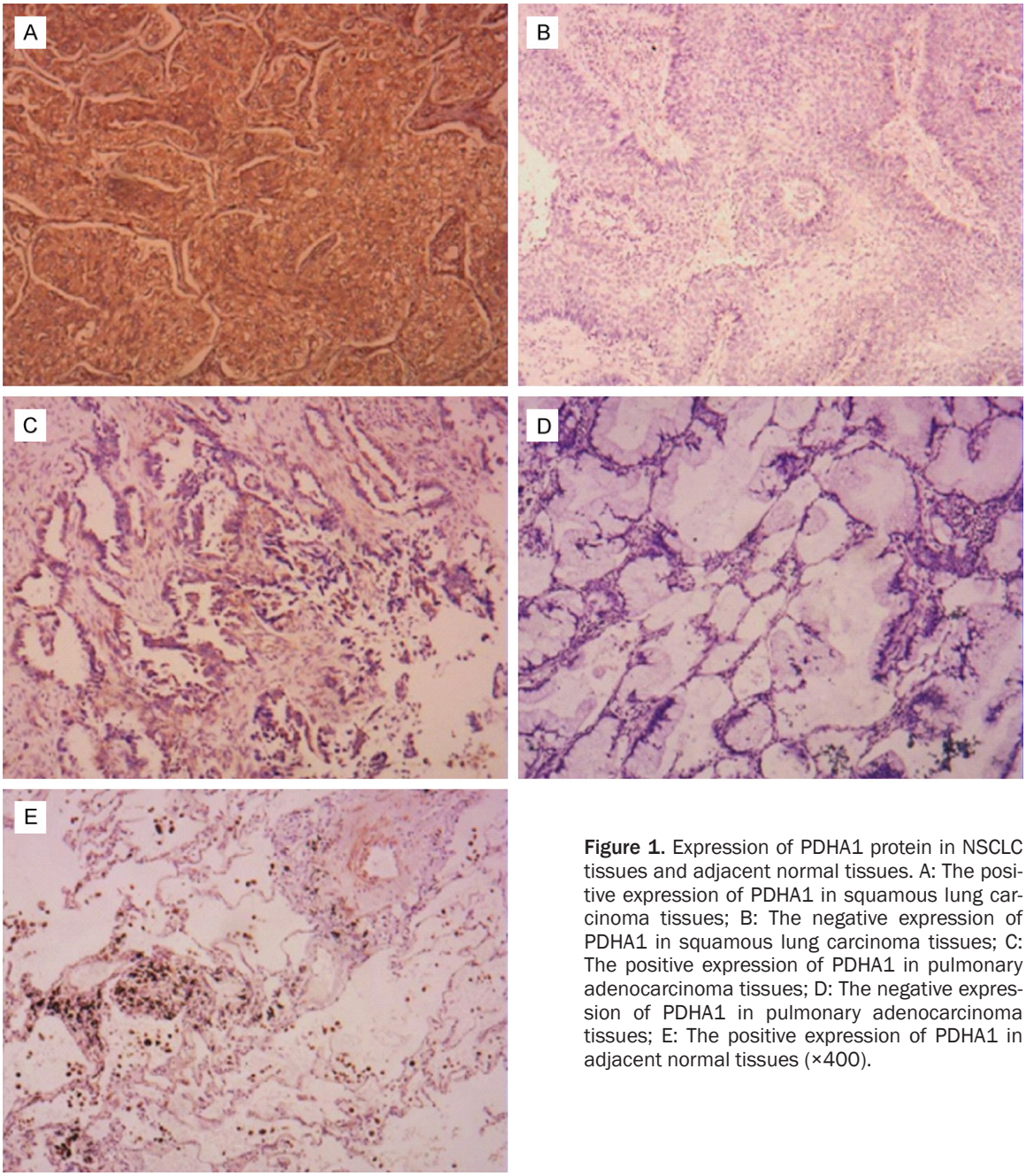

Figure 1. Expression of PDHA1 protein in NSCLC tissues and adjacent normal tissues. A: The positive expression of PDHA1 in squamous lung carcinoma tissues; B: The negative expression of PDHA1 in squamous lung carcinoma tissues; C: The positive expression of PDHA1 in pulmonary adenocarcinoma tissues; D: The negative expression of PDHA1 in pulmonary adenocarcinoma tissues; E: The positive expression of PDHA1 in adjacent normal tissues (×400).

comparison between groups were conducted by Log-rank test and Breslow test. Cox's multivariate proportional hazards regression model was applied to verify the factors affecting patients' OS. $P < 0.05$ was considered statistically significant.

Results

Clinical materials

A total of 196 cases were enrolled in this study, 87 (44.4%) with squamous cell carcinoma, and 109 (55.6%) with adenocarcinoma. It was comprised of 145 males and 51 females. Male-to-female ratio was 2.84:1. Age of onset ranged from 31 to 79, and the median age was 49. The diameter of tumor ranged from 1.5 cm to 11 cm (mean: 4.6 cm, median: 3.9 cm). Median survival time was 39 months (3-62 months).

Expression of PDHA1 protein tested by immunohistochemistry

Expressions of PDHA1 protein in NSCLC tissues and adjacent normal tissues are shown in

Table 1. The correlation between the expression of PDHA1 protein in NSCLC and clinicopathological characteristics

Clinicopathological characteristics	Cases	PDHA1 protein			
		+	-	χ^2	P
Gender					
Male	145	58	87	0.352	0.553
Female	51	18	33		
Pathological type					
Squamous cell carcinoma	87	32	55	0.262	0.609
Adenocarcinoma	109	44	65		
Smoking history					
With	153	62	91	0.879	0.344
Without	43	14	29		
Differentiation degree					
High	41	25	16	17.930	0.000
Intermediate	108	43	65		
Low	47	8	39		
Lymphatic metastasis					
Yes	91	27	64	5.932	0.015
No	105	49	56		
TNM staging					
Stage I	57	29	28	6.619	0.037
Stage II	87	33	54		
Stage III and IV	52	14	38		

Figure 1. The positive expression rate of PDHA1 protein in NSCLC tissues was 38.8% (76/196), while in corresponding adjacent normal tissues was 96.0% (96/100). It indicated that the positive expression rate of PDHA1 protein in NSCLC tissues was significant lower than that of adjacent normal tissues (χ^2=4.144, P=0.018).

Correlation between the expression of PDHA1 protein in NSCLC and the clinicopathological characteristics

This study showed that in NSCLC tissues, the expression of PDHA1 protein had nothing to do with patients' gender, smoking history or pathological type, but was related to differentiation degree of tissues, lymphatic metastasis and TNM staging. The positive expression rate of PDHA1 protein in high differentiated patients was 61.0%, in intermediate differentiated ones was 39.8%, in low differentiated ones was 17.0%, in patients without lymphatic metastasis was 46.7%, in those with lymphatic metastasis was 30%. The positive expression rate of PDHA1 protein in patients was 50.9% in stage I, 37.9% in stage II, 26.9% in stage III-IV. The differences were all statistically significant (P < 0.05 or P < 0.01, see **Table 1**).

Follow-up and survival analysis

The OS rate of the 196 cases was 67.3%, ranged from 3 months to 62 months (still alive), with an average of 37 months and a median of 39 months. The survival analysis suggested that according to the between-group comparison conducted by Log-rank test and Breslow test, the OS rates of PDHA1 positive group and negative group were 78.9% and 60.0%, respectively. The difference was statistically significant (Log-rank, χ^2=21.100, P=0.000; Breslow, χ^2=30.177, P=0.000). The impact of tumor differentiation degree on prognosis manifested that the OS rates of patients in high, intermediate and low differentiation group were 87.8%, 83.3% and 12.8%, respectively. Patients in different differentiation degrees had significant differences in survival time (Log-rank, χ^2=178.270, P=0.000; Breslow, χ^2=30.177, P=0.000). The OS rates of the groups with and without lymphatic metastasis were 47.3% and 84.8%, respectively. It indicated that lymphatic metastasis caused a significant difference in patients' OS rate (Log-rank, χ^2=39.175, P=0.000; Breslow, χ^2=36.897, P=0.000). The OS rates of patients in stage I, II and III-IV were 86.0%, 78.2% and 28.3%, respectively. There were significant differences in patients at different TNM stages (Log-rank, χ^2=96.379, P=0.000; Breslow, χ^2=83.756, P=0.000), as shown in **Figure 2**. Cox's multivariate factors regression analyses revealed the regression coefficient of PDHA1 (B=2.007, P=0.000), which could be considered that patients with positive expression of PDHA1 had comparatively longer survival time in the standard of P < 0.05. On average, the mortality rate of patients with negative PDHA1 was 7.437 times higher than those with positive PDHA1. Besides, the prognosis of NSCLC was related with the differentiation degree of tissues, lymphatic metastasis and TNM staging (P < 0.001, **Table 2**).

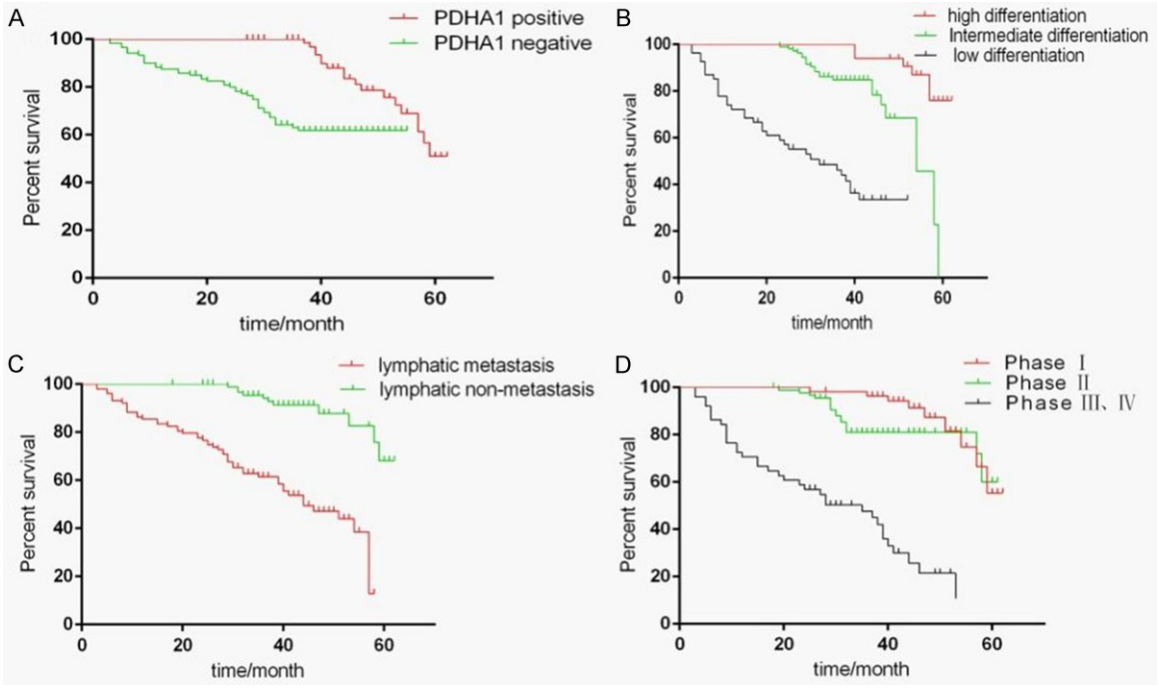

Figure 2. Impact of clinicopathological characteristics on the OS of NSCLC. A: The impact of positive/negative expression of PDHA1 protein on the OS of NSCLC; B: The impact of differentiation degree on the OS of NSCLC; C: The impact of lymphatic metastasis on the OS of NSCLC; D: The impact of TNM staging on the OS of NSCLC.

Table 2. Multivariate factors regression analyses of the prognosis of NSCLC

Variables	B	SE	Wald	df	Sig.	Exp (B)
Gender	-14.519	110.659	0.017	1	0.896	0.000
Pathological type	0.210	0.291	0.517	1	0.472	1.233
Smoking history	1.007	0.544	3.462	1	0.064	2.737
DD	1.336	0.277	24.272	1	0.000	3.919
LM	-1.773	0.385	20.232	1	0.000	0.177
TNM staging	1.010	0.254	15.775	1	0.000	2.747
PDHA1	2.007	0.498	16.240	1	0.000	7.473

Note: DD: differentiation degree; LM: lymphatic metastasis.

Discussion

Pyruvate dehydrogenase complex (PDC) is a kind of multienzyme complex that can catalyze pyruvate decarboxylation reactions. PDC, which exists extensively in mammals' mitochondrial matrix, is composed of pyruate dehydrogenase (PDH), dihydrolipoamide acetyltransferase (E2) and dihydrolipoamide dehydrogenase (E3) [5-7]. PDHA1 is one of the vital subunits of PDH, and its normal expression is the premise of normal tricarboxylic acid cycle and oxidative phosphorylation in mitochondrion. Inhibition of PDH can restrain the oxidative phosphorylation of mitochondrion and consequently improve the aerobic glycolysis of tumor [8, 9]. In recent years, a large number of studies have demonstrated that PDHA1 can facilitate the growth and metastasis of tumor by affecting its metabolic phenotype [10, 11]. Meanwhile, some studies have manifested that fasting and metabolic pathway remodeling of tumor can not only enhance the apoptosis of tumor cells, but also inhibit the resistance of tumor cells to chemotherapeutic drugs [12]. Rajagopalan et al. applied a genetic approach to perform the PDHA1 gene knockout on tumor cells, and they found that the deletion of PDHA1 gene could enhance the cell proliferation [13]. Besides, PDHA1 gene also had a certain association with apoptosis caused by serial reactions of respiratory chain activity, generation of reactive oxygen species and the regulation of caspase in the mitochondrion of tumor cells [14].

However, the expression of PDHA1 and its impact on NSCLC are not clear yet. In this study, the expressions of PDHA1 protein in 196

NSCLC tissues and adjacent normal tissues were immunohistochemically detected and the correlation between the expression of PDHA1 protein and the clinicopathological characteristics of NSCLC was first examined. The results suggested that expression of PDHA1 protein had no relationship with patients' gender, smoking history and pathological types, but it was related to differentiation degree of tissues, lymphatic metastasis and TNM staging. To be more specific, the expression of PDHA1 protein in patients with high and intermediate differentiation was higher compared with low differentiated ones. Patients without lymphatic metastasis showed higher expression of PHDA1 protein than those with it. Besides, patients in stage I and II had higher expression of PDHA1 protein than those in stage III and IV. These evidences basically meant that the low expression of PDHA1 was concerned with the poor prognoses of NSCLC. The study, conducted by Hamabe et al., indicated that hexokinase 2 (HK2) and PDHA1 had crucial impacts on the prognoses of patients with colon cancer [15]. The tumor cells of patients with positive HK2 and negative PDHA1 had stronger invasion ability, which led to the worse prognoses. And these results were in consistent with our study. Both studies showed that the positive PDHA1 was a protective factor for the prognoses of patients with tumor. But when Hamabe et al. only detected the correlation between the PDHA1 expression and the colon cancer patients' clinicopathological characteristics, they drew a conclusion that the expression of PDHA1 had certain impacts on the tumor size, metastasis, invasion and clinical staging. However, the impacts had no significant difference ($P > 0.05$). There were some differences between their study and ours in this point, which may due to the tumor types and clinical samples. Liu et al. found that the hepatitis B X-interacting protein (HBXIP) could reconstruct the metabolic pathway of mammary cancer cells by down-regulating the cytochrome c oxidase 2 (SCO2) and PDHA1, and thus promoted the proliferation of mammary cancer cells [16]. It also reflected that the down-regulation or low expression of PDHA1 could promote the occurrence and development of tumor.

In this study, the impact of PDHA1 on the OS of patients with NSCLC had been detected. The results showed that the OS rate of 196 patients with NSCLC was 67.3%. The survival rate was 78.9% in positive PDHA1 protein group, and 60.0% in negative PDHA1 protein group, with a significant difference. Besides, the OS was comparatively longer in patients with high differentiation, without lymphatic metastasis and patients in stage I. At last, in this study, the Cox's multivariate factors regression analysis verified that the expression of PDHA1, tissue differentiation degree, lymph node metastasis and TNM staging had vital impacts on the prognosis of NSCLC. The systemic inquiry of 248 patients with ovarian cancer conducted by Li et al. showed that the low expression of PDHA1 was significantly related to the poor prognoses of patients with ovarian cancer [2]. The patients with positive PDHA1 expression had longer OS, which was similar to the results of this study.

Hypoxia is one of the basic characteristics of the microenvironment of most tumors, especially the solid tumors. After inhibiting PI3K/AKT signaling pathway and causing the hypoxic-micro environment of SA20B tumor cell line, George et al. found that hypoxia could promote the Ser phosphorylation in PDHA1 293 bit, and further restrained the activity of PDH and then significantly decreased the proliferation of SQ20B [17]. These findings further affirmed the correlation between PDHA1 expression and good prognosis from the mechanism level. However, the expression of PDH in NSCLC tumor cells is relatively absent, and the main cause of the loss hasn't been fully elucidated yet. The absence of PDH results in the excessive accumulation of pyruvic acid in NSCLC, thereby leading to the up-regulation of HIF-α [6, 18]. The up-regulation of HIF-α can not only promote the proliferation, metastasis and invasion of tumor cells and the formation of the tumor microvessel [19], but also inhibit the expression of PDHA1 by regulating various miRNA [6]. It also implies that PDHA1 has an important influence on NSCLC. The latest researches proved that the deletion of PDHA1 could make the clinical prognoses of prostate cancer patients obviously worse than those with the expression of PDHA1 [20]. The main mechanism was that the deletion of PHDA1 gave the tumor cells some phenotypes of pluripotent stem cells, thus these tumor cells had stronger proliferation and invasion abilities, and were more resistant to the chemotherapeutic drugs.

To some extent, it also explained the mechanism of the drug resistance of tumor.

This study explored the expression of PDHA1 in NSCLC and its connection with OS. The result indicates that the decreased expression of PDHA1 can promote tumor progression and cause a poor prognosis. However, in this study, its mechanism is not verified yet and it is speculated that the mechanism may be related to the high expression of HIF-α in NSCLC and the alteration of tumor cell phenotypes. On the basis of this study, further inquiry about the exact function of PDHA1 in NSCLC and the related mechanisms should be done by means of PDHA1 knockout and overexpression [21]. In conclusion, this studyis indicative of a negative correlation between the expression of PDHA1 in NSCLC and the clinicopathological characteristics. Moreover, PDHA1, as a good marker in NSCLC, is important for the clinical evaluation of patients' prognoses.

Disclosure of conflict of interest

None.

Address correspondence to: Zhenhe Suo, Department of Oncology, The First Affiliated Hospital of Zhengzhou University; Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, University of Oslo, Montebello, Oslo 0379, Norway; Department of Pathology, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo 0379, Norway. Tel: +86-13526727345; E-mail: suozhnw@126.com

References

[1] Travis WD, Travis LB and Devesa SS. Lung cancer. Cancer 1995; 75: 191-202.

[2] Li Y, Huang R, Li X, Li X, Yu D, Zhang M, Wen J, Goscinski MA, Trope CG, Nesland JM and Suo Z. Decreased expression of pyruvate dehydrogenase A1 predicts an unfavorable prognosis in ovarian carcinoma. Am J Cancer Res 2016; 6: 2076-2087.

[3] Li Y, Li X, Li X, Zhong Y, Ji Y, Yu D, Zhang M, Wen JG, Zhang H, Goscinski MA, Nesland JM and Suo Z. PDHA1 gene knockout in prostate cancer cells results in metabolic reprogramming towards greater glutamine dependence. Oncotarget 2016; 7: 53837-53852.

[4] Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC and Harris AL. Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. Neoplasia 2005; 7: 1-6.

[5] Patel MS, Nemeria NS, Furey W and Jordan F. The pyruvate dehydrogenase complexes: structure-based function and regulation. J Biol Chem 2014; 289: 16615-16623.

[6] Patel MS and Korotchkina LG. Regulation of mammalian pyruvate dehydrogenase complex by phosphorylation: complexity of multiple phosphorylation sites and kinases. Exp Mol Med 2001; 33: 191-197.

[7] Korotchkina LG and Patel MS. Binding of pyruvate dehydrogenase to the core of the human pyruvate dehydrogenase complex. FEBS Lett 2008; 582: 468-472.

[8] Sun XR, Sun Z, Zhu Z, Guan HX, Li CY, Zhang JY, Zhang YN, Zhou H, Zhang HJ, Xu HM and Sun MJ. Expression of pyruvate dehydrogenase is an independent prognostic marker in gastric cancer. World J Gastroenterol 2015; 21: 5336-5344.

[9] Costello LC and Franklin RB. 'Why do tumour cells glycolyse?': from glycolysis through citrate to lipogenesis. Mol Cell Biochem 2005; 280: 1-8.

[10] Kim JW, Tchernyshyov I, Semenza GL and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006; 3: 177-185.

[11] Dupuy F, Tabaries S, Andrzejewski S, Dong Z, Blagih J, Annis MG, Omeroglu A, Gao D, Leung S, Amir E, Clemons M, Aguilar-Mahecha A, Basik M, Vincent EE, St-Pierre J, Jones RG and Siegel PM. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metab 2015; 22: 577-589.

[12] Bianchi G, Martella R, Ravera S, Marini C, Capitanio S, Orengo A, Emionite L, Lavarello C, Amaro A, Petretto A, Pfeffer U, Sambuceti G, Pistoia V, Raffaghello L and Longo VD. Fasting induces anti-Warburg effect that increases respiration but reduces ATP-synthesis to promote apoptosis in colon cancer models. Oncotarget 2015; 6: 11806-11819.

[13] Rajagopalan KN, Egnatchik RA, Calvaruso MA, Wasti AT, Padanad MS, Boroughs LK, Ko B, Hensley CT, Acar M, Hu Z, Jiang L, Pascual JM, Scaglioni PP and DeBerardinis RJ. Metabolic plasticity maintains proliferation in pyruvate dehydrogenase deficient cells. Cancer Metab 2015; 3: 7.

[14] Glushakova LG, Lisankie MJ, Eruslanov EB, Ojano-Dirain C, Zolotukhin I, Liu C, Srivastava A and Stacpoole PW. AAV3-mediated transfer and expression of the pyruvate dehydrogenase E1 alpha subunit gene causes metabolic remodeling and apoptosis of human liver cancer cells. Mol Genet Metab 2009; 98: 289-299.

[15] Hamabe A, Yamamoto H, Konno M, Uemura M, Nishimura J, Hata T, Takemasa I, Mizushima T, Nishida N, Kawamoto K, Koseki J, Doki Y, Mori M and Ishii H. Combined evaluation of hexokinase 2 and phosphorylated pyruvate dehydrogenase-E1alpha in invasive front lesions of colorectal tumors predicts cancer metabolism and patient prognosis. Cancer Sci 2014; 105: 1100-1108.

[16] Liu F, Zhang W, You X, Liu Y, Li Y, Wang Z, Wang Y, Zhang X and Ye L. The oncoprotein HBXIP promotes glucose metabolism reprogramming via downregulating SCO2 and PDHA1 in breast cancer. Oncotarget 2015; 6: 27199-27213.

[17] Cerniglia GJ, Dey S, Gallagher-Colombo SM, Daurio NA, Tuttle S, Busch TM, Lin A, Sun R, Esipova TV, Vinogradov SA, Denko N, Koumenis C and Maity A. The PI3K/Akt Pathway Regulates Oxygen Metabolism via Pyruvate Dehydrogenase (PDH)-E1alpha Phosphorylation. Mol Cancer Ther 2015; 14: 1928-1938.

[18] Lu H, Forbes RA and Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J Biol Chem 2002; 277: 23111-23115.

[19] Zurlo G, Guo J, Takada M, Wei W and Zhang Q. New insights into protein hydroxylation and its important role in human diseases. Biochim Biophys Acta 2016; 1866: 208-220.

[20] Zhong Y, Li X, Ji Y, Li X, Li Y, Yu D, Yuan Y, Liu J, Li H, Zhang M, Ji Z, Fan D, Wen J, Goscinski MA, Yuan L, Hao B, Nesland JM and Suo Z. Pyruvate dehydrogenase expression is negatively associated with cell stemness and worse clinical outcome in prostate cancers. Oncotarget 2017; 13344-13356.

[21] Han Z, Gorbatyuk M, Thomas J Jr, Lewin AS, Srivastava A and Stacpoole PW. Down-regulation of expression of rat pyruvate dehydrogenase E1alpha gene by self-complementary adeno-associated virus-mediated small interfering RNA delivery. Mitochondrion 2007; 7: 253-259.