Oncology Research, Vol. 27, pp. 407–414
Printed in the USA.

0965-0407/19 $90.00 + .00
DOI: https://doi.org/10.3727/096504018X15180451872087
E-ISSN 1555-3906
www.cognizantcommunication.com

Overexpression of Pyruvate Dehydrogenase E1α Subunit Inhibits Warburg Effect and Induces Cell Apoptosis Through Mitochondria-Mediated Pathway in Hepatocellular Carcinoma

Jihong Sun,*†[1] Jingjing Li,†[1] Zhixian Guo,† Lu Sun,†‡ Chenghui Juan,§ Yubing Zhou,¶ Hongli Gu,† Yan Yu,† Qiuyue Hu,† Quancheng' Kan,¶ and Zujiang Yu†

*Department of Internal Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China
†Department of Infectious Disease, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China
‡Department of Gastroenterology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China
§Department of Otolaryngology Head and Neck Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China
¶Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China

Most cancers rely disproportionately on glycolysis for energy even in the presence of an adequate oxygen supply, a condition known as "aerobic glycolysis," or the "Warburg effect." Pyruvate dehydrogenase E1 subunit (PDHA1) is one of the main factors for the metabolic switch from oxidative phosphorylation (OXPHOS) to aerobic glycolysis and has been suggested to be closely associated with tumorigenesis. Here we observed that the PDHA1 protein was reduced in hepatocellular carcinoma (HCC) specimens by immunohistochemistry and Western blot, which was significantly associated with poor overall survival. To further analyze the function of PDHA1 in cancer cells, PDHA1 was upregulated in the HCC cell lines SMMC-7721 and HepG2. The results demonstrated that overexpression of the PDHA1 gene inhibited aerobic glycolysis with lower lactate via increased PDH activity; meanwhile, mitochondrial OXPHOS was enhanced accompanied with higher ATP and lower glucose consumption. We also found that apoptosis was promoted and intrinsic pathway proteins were increased in PDHA1-overexpressing cells. Collectively, our data indicate that reduced PDHA1 protein expression is associated with the poor clinical outcome of HCC. Upregulated PDHA1 gene expression can inhibit the Warburg effect and enhance the mitochondria-mediated apoptosis pathway.

Key words: Pyruvate dehydrogenase E1α subunit (PDHA1); Warburg effect; Apoptosis; Hepatocellular carcinoma (HCC)

INTRODUCTION

The Warburg effect, also known as "aerobic glycolysis," refers to the phenomenon that cancer cells preferentially utilize glycolysis over oxidative phosphorylation (OXPHOS) even in the presence of sufficient oxygen to support mitochondrial respiratory function[1]. Such alterations in cellular metabolism may favor tumor cell growth by increasing the availability of the biosynthetic intermediates needed for cellular growth and proliferation[2]. Increased aerobic glycolysis is a widely observed feature in human cancers and often correlates with tumor aggressiveness and poor patient prognosis in many tumor types, including human hepatocellular carcinoma (HCC), the main type of liver cancer[3–6]. So a better understanding of the mechanistic link between cell metabolism and survival control could be of paramount significance for the development of new therapeutics in HCC[2–5].

Emerging evidence has revealed that aerobic glycolysis supported cell survival and growth through apoptosis resistance[7,8]. Bianchi et al.[9] found that glucose-deficient conditions could promote an anti-Warburg effect characterized by increased oxygen consumption but a failure to generate adenosine-5 -triphosphate **(**ATP), resulting in oxidative damage and apoptosis. It is therefore advocated that inhibition of the Warburg effect can induce apoptosis through metabolic changes, thereby inhibiting cell growth.

As we know, the pyruvate dehydrogenase complex (PDH) is the most important enzyme that transforms

[1]These authors provided equal contribution to this work.
Address correspondence to Quancheng' Kan, M.D., Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Room 120, 9th Building, No. 1 Jian-She East Road, Zhengzhou 450052, P.R. China. E-mail: quancheng_k@163.com *or* Zujiang Yu, M.D., Department of Infectious Disease, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, P.R. China. E-mail: zuj_yu@163.com

pyruvate into acetyl-CoA, which then enters the TCA cycle to produce ATP and electron donors. PDH is composed of the E1, E2, and E3 components. Pyruvate decarboxylation is catalyzed by E1, and this is considered to be the rate-limiting step[10]. E1 is a heterotetramer of two and two subunits, with the E1 subunit encoded by the PDHA1 gene[11]. As the main active subunit of PDH, PDHA1 could be one of the most important proteins involved in aerobic glycolysis. Inhibition of PDHA1 has been shown to decrease mitochondrial OXPHOS and promote tumor aerobic glycolysis in tumor cells[12]. To examine the relationship between the glucose metabolism switch and apoptosis in cancer cells, we chose PDHA1 for further studies.

In our study, the expression of PDHA1 in a series of tumor tissues from HCC patients was first verified, then its correlations with clinical pathological parameters and overall survival (OS) were examined. Subsequently, the *PDHA1* gene was upregulated using a lentivirus infection assay, and the glycolysis features and apoptosis in HCC cells were then studied in comparison to the parental cells.

MATERIALS AND METHODS

Clinical Samples

In this study, two sets of HCC samples were used. First, the HCC tissue microarray (TMA; Shanghai Outdo Biotech Co., Ltd, Shanghai, P.R. China) containing 75 cases of HCC samples was used to compare the expression of PDHA1 between tumor tissues and corresponding noncancerous liver (CNL) tissues. Then we estimated a relationship between the expression of PDHA1 and clinicopathological features. The second set was used for identification of the *PDHA1* messenger RNA (mRNA) using quantitative real-time PCR (RT-qPCR) and protein expression levels by Western blot. The second set of HCC specimens was obtained from patients who underwent surgical resection of their tumors and signed an informed consent before their liver operations in the Department of General Surgery of The First Affiliated Hospital of Zhengzhou University (Zhengzhou, Henan, P.R. China). All procedures performed in studies involving human participants were reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University. Informed consent was obtained from all individual participants included in the study.

Cell Line and Cell Culture

Human hepatoma cell lines SMMC-7721 and HepG2 were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, P.R. China) and maintained in our laboratory. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Solarbio, Beijing, P.R. China) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified 5% CO_2 incubator.

Lentivirus Infection Assay

Lentivirus plasmids were constructed by GenePharma (Shanghai, P.R. China). The LV5-PDHA1-homo infection can upregulate the expression of PDHA1. For infection, the HCC cell lines SMMC-7721 and HepG2 at 50%–80% confluence were transfected with the viral suspension according to the manufacturer's instructions, and the stable cell line (PDHA1 UP) was established with lentivirus selected with puromycin according to the manufacturer's instruction. Cells were harvested after infection for 48 h, and the infected target cells were identified by RT-qPCR and Western blot.

Western Blot and Antibodies

Cell or tissue lysate was separated on an 8% or 10% SDS-PAGE and transferred to nitrocellulose membrane under constant current condition. Then the membrane was blocked with 5% nonfat milk at normal temperature for 1 h. The nitrocellulose membrane was then incubated with indicated antibody at 4°C overnight. The next day, the nitrocellulose membrane was incubated with the fluorescence-conjugated secondary antibodies at room temperature for 1 h. The images of the Western blot were analyzed using ImageJ software for gray value calculation. Rabbit polyclonal anti-PDHA1, anti-GAPDH, cleaved caspases 3 and 9, Bcl-2, Bax, and cytochrome c (cyto C) antibody were all purchased from Cell Signaling Technology (Boston, MA, USA). Caspase 3 and 9 were from Proteintech Group, Inc (Wuhan, P.R. China). Secondary horseradish peroxidase-conjugated antibodies were goat anti-mouse and goat anti-rabbit from Sigma-Aldrich Corporation (St. Louis, MO, USA).

RT-qPCR

Total RNA was extracted using the TRIzol reagent according to the manufacturer's protocol (Takara, Dalian, P.R. China). RNA samples (1 μg) were reverse transcribed to generate first-strand cDNA. RT-qPCR was performed in a 20-μl reaction mixture prepared with SYBR GREEN PCR Master Mix (Applied Biosystems, Foster City, CA, USA) containing an appropriately diluted cDNA solution and 0.2 M of each primer. The program consisted of 95°C for 10 min, followed by 35 cycles at 95°C for 10 s, and 60°C for 45 s. The transcript levels of PDHA1 were detected by RT-qPCR analysis using a StepOne system (CFX96 Touch PCR; Bio-Rad, Hercules, CA, USA). All reactions were conducted in triplicate, and the data were analyzed using the Ct ($2^{-\ Ct}$) method. These transcripts were normalized to -actin.

CCK-8 Cell Proliferation Assay

The infected cells were seeded into 96-well plates at a density of 2,000 cells per 100 µl and cultured for 5 days. Ten microliters of CCK-8 reagent (TransGen Biotech, Beijing, P.R. China) was added to each well and reacted for 2 h under light-free conditions, at 37°C, and in 5% CO_2 atmosphere. Then the absorbance value was detected using a microplate reader. The absorbance value at 450 nm was recorded.

Colony Formation Assay

The infected cells were seeded in six-well plates (0.5×10^3 cells per well) and cultured for 2 weeks. Colonies were fixed with paraformaldehyde for 10 min and stained with 1% crystal violet (Sigma-Aldrich) for 15 min, and then the colonies were counted. The rate of colony formation from each group was analyzed. Triplicate independent experiments were carried out.

Apoptosis Assay

Cell apoptosis was detected using an Annexin-V–allophycocyanin (APC)/propidium iodide (PI) staining kit (Roche, Basel, Switzerland). HepG2 and SMMC-7721 cells were collected and stained with annexin V and suspended in 500 µl of annexin V-binding buffer. Then the cells were incubated with 5 µl of annexin V–APC for 15 min in the dark at room temperature. Then 5 µl of PI was added to the mixture and passed through a 300-mesh sieve. The samples were analyzed by flow cytometry utilizing a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). Each group was measured in triplicate.

PDH Enzymatic Activity Assay

A PDH activity assay kit (BioVision, San Francisco, CA, USA) was utilized to measure PDH activity according to the manufacturer's instructions. PDH catalyzes the reaction of pyruvic acid to produce a reductive intermediate product, NADH, which can generate a substance that has strong absorption at a wavelength of 450 nm. The activity of PDH was reflected by measuring the change of absorption at 450 nm.

Determination of Glucose Consumption, Lactate, and ATP Production

Cells (1×10^6 cells per well) were seeded and cultured for 24 h. The cell culture medium was tested for glucose consumption and lactate assay using a glucose assay kit (Sigma-Aldrich) and a lactate assay kit (BioAssay Systems, Hayward, CA, USA), respectively, according to the manufacturer's instructions. For cellular ATP production determination, 1×10^6 cells were collected, and cell lysis was achieved by sonication (in the ice-water bath) before cellular ATP was detected with a Molecular Probes' ATP Determination Kit (Life Technology, Shanghai, P.R. China) according to the manufacturer's instructions.

Statistical Analysis

All statistical analyses were performed using the SPSS software (version 17.0; SPSS Statistic, Chicago, IL, USA). The data are expressed as the mean ± standard deviation from separate experiments and analyzed using the Student's *t*-test. Paired *t*-test was used for paired samples. A value of $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

PDHA1 Was Downregulated in HCC Tissues and Correlated With Poor Prognosis in HCC Patients

Expression levels of PDHA1 were compared using RT-qPCR and Western blot in 15 pairs of HCC/CNL tissues. The results showed that the expression of PDHA1 was significantly lower in HCC tissues than in CNL tissues (Fig. 1A and B). To estimate the association of PDHA1 with tumor biological behaviors, we next examined the comparison of the clinical pathological features with PDHA1 expression by TMA. The immunohistochemistry experiment showed that PDHA1 was dominantly expressed in the cytoplasm of HCC tissues in contrast to the adjacent normal tissues (Fig. 1C). Density analysis of TMA also showed that PDHA1 expression was downregulated in 69.33% (52/75) of HCC patients ($p < 0.05$). The results demonstrated that PDHA1 expression correlated with tumor size, tumor differentiation, and TNM stage. There was no significant difference in age, gender, and liver cirrhosis ($p > 0.05$). Kaplan–Meier analysis reflected that a reduced level of PDHA1 expression was associated with poor OS (Fig. 1D). All the results above revealed that the expression of PDHA1 was decreased in HCC tissues, compared to CNL tissues, at both protein and mRNA levels, and also demonstrated that loss of, or low PDHA1, expression might be considered as a marker of tumor aggressiveness, and decreased expression of PDHA1 in HCC was predictive of unfavorable outcomes.

PDHA1 Gene Overexpression Could Decrease PDH Activity and Change Cell Metabolic Patterns

We induced overexpression of PDHA1 in SMMC-7721 and HepG2 cells using the lentivirus infection assay and identified the infection effect using qPCR and Western blot assay (Fig. 2A). Since the Warburg effect (or aerobic glycolysis) is considered a common metabolic phenomenon in cancer cells, we measured PDH activity, glucose consumption, and lactic acid and ATP production in SMMC-7721 and HepG2 cells after upregulation of PDHA1 (PDHA1 UP) to investigate the effect of PDHA1

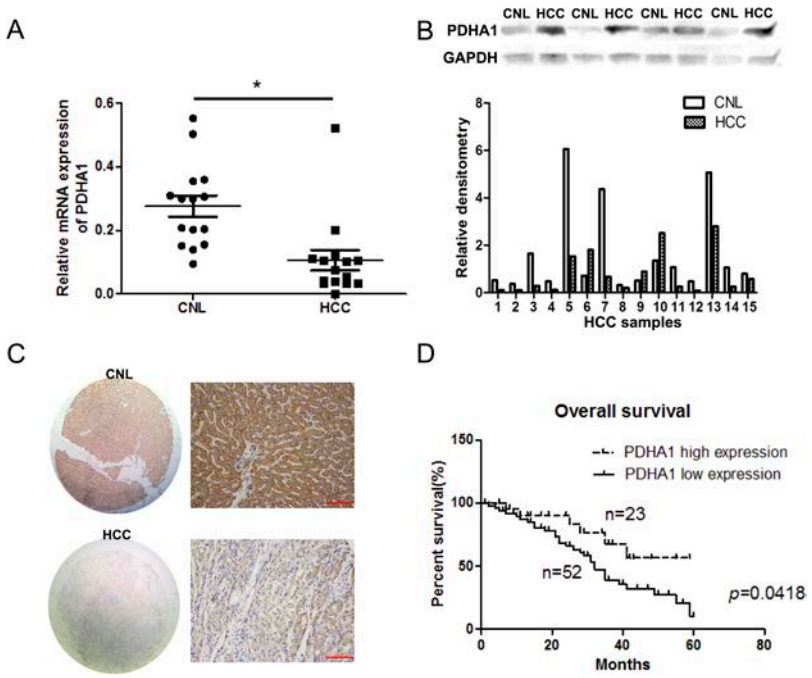

Figure 1. Expression of PDHA1 was downregulated in HCC and associated with poor patient clinical outcomes. Expression levels of PDHA1 in 15 pairs of HCC/CNL tissues were measured by RT-qPCR (A) and Western blot (B). (C) Immunohistochemical staining of PDHA1 in paired HCC and CNL tissues. (D) Association of PDHA1 expression with overall survival calculated by the Kaplan–Meier method and analyzed by the log-rank test. *$p<0.05$, compared to the NC group, according to the two-tailed Student's *t*-test. Abbreviations: CNL, corresponding noncancerous liver; HCC, hepatocellular carcinoma; NC, negative control; PDHA1, pyruvate dehydrogenase E1 subunit; RT-qPCR, quantitative real-time polymerase chain reaction.

on the Warburg effect. Our data showed that PDH activity was significantly higher (Fig. 2B) in PDHA1 UP cells than in negative control (NC) cells. We also found that PDHA1 UP cells exhibited an increase in ATP production (Fig. 2D), but a decrease in lactate production (Fig. 2E) and glucose uptake (Fig. 2C), which are hallmarks of glycolysis. These results support the notion that the Warburg effect was suppressed when PDHA1 gene expression was upregulated.

Overexpression of PDHA1 Inhibited the Growth of HCC Cells In Vitro

The CCK-8 assay directly reflects cell viability and can be used for evaluating cell proliferation. We collected SMMC-7721 and HepG2 cells infected with lentivirus for 48 h and detected cell viability for 5 days in vitro using the CCK-8 assay. The results showed that the proliferative capacity in PDHA1 UP cells was remarkably poorer than in NC cells (Fig. 3A). Cell cloning experiments also showed the same tendency (Fig. 3B).

Apoptosis of Mitochondrial Pathway Was Activated in PDHA1-Overexpressing Cells

The rates of apoptosis were assessed. Cells undergoing early stage apoptosis and late stage apoptosis were considered as apoptotic cells. The results showed that the apoptotic rates increased in PDHA1 UP cells (Fig. 4A and B). We tested associated proteins of the mitochondrial apoptotic pathway for underlying mechanisms of apoptosis (Fig. 4C). PDHA1 UP cells showed an elevation of Bax protein and a reduced expression of Bcl-2 protein. The result also indicated that the expression of cleaved caspases 3 and 9 and cytosol cyto C was significantly increased. The data demonstrated that overexpression of the PDHA1 gene could improve mitochondrial apoptotic protein expression, inducing downregulation of antiapoptotic protein Bcl-2 and upregulation of the proapoptotic protein Bax.

DISCUSSION

Our results showed that PDHA1 was lowly expressed in HCC tissues compared to adjacent normal tissues, and low PDHA1 protein expression was related to poor clinical outcome in HCC patients. The next study demonstrated that overexpression of the PDHA1 gene downregulated glycolysis and promoted OXPHOS. We also found that upregulation of the PDHA1 gene could increase intrinsic cell apoptosis.

In human HCC tissues, we showed that PDHA1 was lowly expressed. Further analysis reflected that reduced

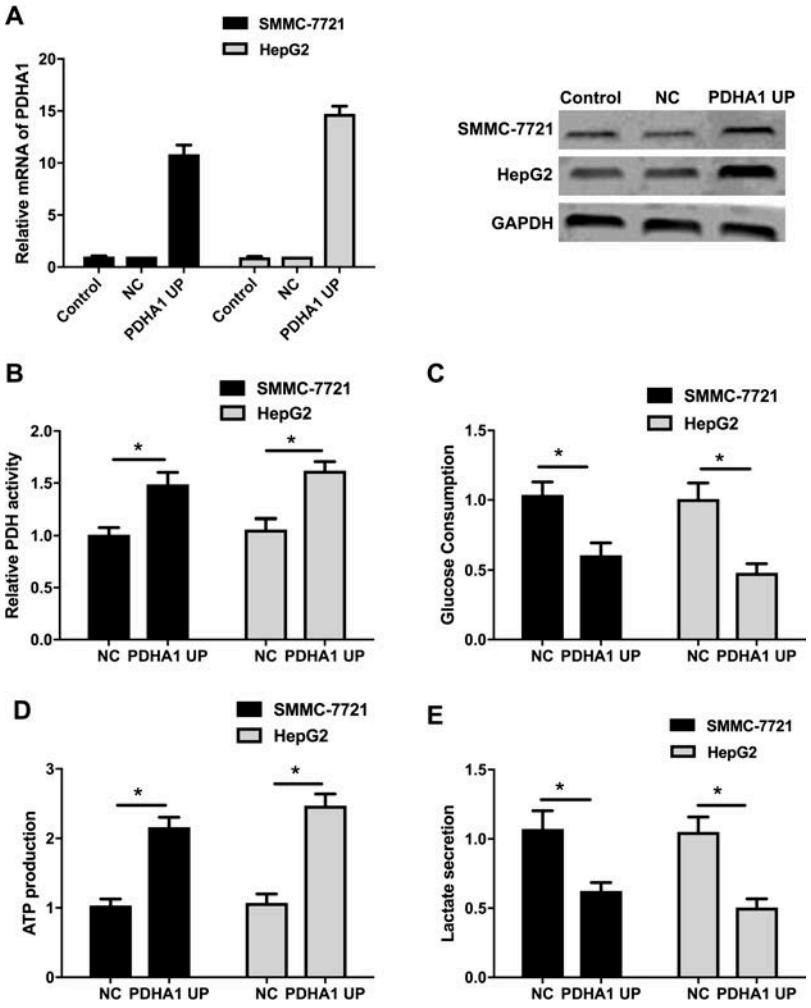

Figure 2. The upregulation of PDHA1 gene expression significantly influences glucose metabolism in SMMC-7721 and HepG2 cells. (A) Identification of the infection effect using RT-qPCR and Western blot assay. (B) The PDH enzymatic activity assay showed that overexpression of PDHA1 increased PDH activity in SMMC-7721 and HepG2 cells. (C) The results of glucose consumption showed that the cells consumed less glucose in PDHA1 UP cells. (D) The ATP production indicated ATP synthesis was higher when PDHA1 was upregulated in SMMC-7721 and HepG2 cells. (E) The lactate production assay showed that upregulation of the PDHA1 gene blocked lactate secretion. The data are shown as means±standard deviation (SD). *$p<0.05$, compared to the NC group, according to the two-tailed Student's *t*-test. Abbreviations: ATP, adenosine-5 -triphosphate; PDHA1 UP, upregulation of PDHA1 gene.

PDHA1 protein in HCC predicted a shorter OS and higher metastasis ability, which implicated PDHA1 as a marker for HCC aggressiveness and a predictor of HCC survival. The expression of PDHA1 was also correlated with other clinical pathological features of HCC, such as tumor size, tumor differentiation, and tumor stage. These findings were consistent with some studies on prostate cancer and esophageal squamous cell carcinoma[13,14].

The relationship between glycolysis and mitochondrial OXPHOS, which are the two energy metabolism models, is mutual cooperation and competition. One of the remarkable features of cancer cells is aerobic glycolysis, also known as the Warburg Effect, in which cancer cells rely preferentially on glycolysis instead of mitochondrial OXPHOS as the main energy source even in the presence of high oxygen tension. In normal differentiated cells, pyruvate is generated by glucose metabolism, and it mainly enters the TCA cycle of OXPHOS to produce more ATP, while in tumor cells, pyruvate is mainly converted into lactic acid through aerobic glycolysis[9]. The metabolism of pyruvate in different directions can lead to changes in mitochondrial OXPHOS and glycolysis rate[15].

PDH is the first and most important enzyme in the transformation of pyruvate into acetyl-CoA, which enters the TCA cycle to produce ATP and electron donors including NADH. Since PDH catalyzes the rate-limiting step during the pyruvate decarboxylation, the activity

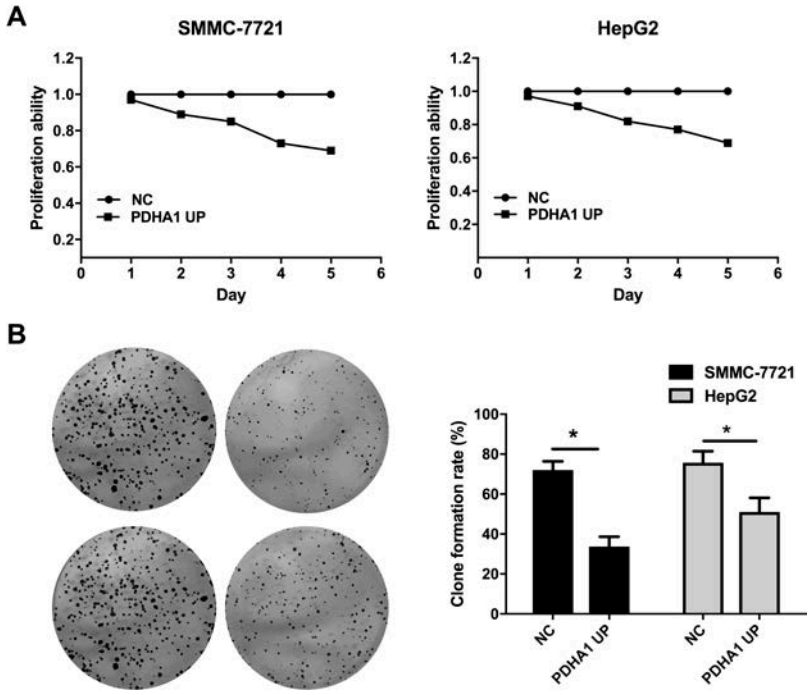

Figure 3. Cell proliferation of HCC cells detected by CCK-8 assay and clone formation assay. The CCK-8 assay was used for cell viability detection and reflected cell proliferation. (A) PDHA1 UP cells showed inhibited proliferation. (B) The results showed that PDHA1 UP cells formed significantly more clones compared to NC cells. *$p < 0.05$, compared to the NC group, according to the two-tailed Student's *t*-test.

of PDH determines the rate of mitochondrial OXPHOS and glycolysis. In the majority of biochemically proven cases, most PDH deficiency arises from mutations in the PDHA1 gene, while mutations are rare in the genes encoding E2, E3, and E3-binding protein[16]. Patel et al.[17] also found that 76% of PDH deficiency were due to a deficit PDHA1 in 371 cases of PDH deficiency, between 1970 and 2010. Lower levels of PDH activity are significantly associated with tumor cell aerobic glycolysis and their malignancy. In PDHA1 UP cells, PDH activity was significantly increased, which indicated that the Warburg effect was inhibited.

Cells generate ATP mainly through mitochondrial OXPHOS. However, the Warburg effect shifts the efficient ATP production from mitochondrial OXPHOS to the significantly less effective ATP production process of aerobic glycolysis, which excretes a large amount of lactate. To fulfill tumor cell needs, the glycolytic switch is associated with elevated glucose uptake[18,19]. In our study, we found that PDHA1 UP cells consumed less glucose and produced less lactate, accompanied with higher generation of ATP. Therefore, we can draw a conclusion that upregulation of the PDHA1 gene can inhibit the Warburg effect through influencing PDH activity.

Apoptosis is a programmed mode of cell death that permits the removal of unwanted cells from the body at a specific time or in response to a given signal. Nutrient, oxygen, and energy supplies have a critical role in signaling a cell to live or die. Recently, it has been recognized that crosstalk between cell metabolism and cell death machinery may have a major contribution in cellular life and death decisions[20,21]. Apoptosis occurs through two main signaling pathways: an extrinsic pathway and an intrinsic pathway, which is also known as the mitochondrial pathway[22]. The mitochondrial pathway of apoptosis is regulated by Bcl-2 family members, which are composed of pro- and antiapoptosis proteins[23]. The proapoptosis proteins mainly include Bax, Bad, and Bak, while antiapoptosis proteins contain Bcl-2, Bcl-XL, etc. A high ratio of Bax to Bcl-2, which causes loss of mitochondrial membrane potential, can facilitate the release of cyto C from the inner mitochondrial membrane to the cytosol. In our study, we found that the cells overexpressed PDHA1, which increased the apoptosis rate, resulting in an elevation of Bax protein and a reduced expression of Bcl-2 protein. The ratio of Bax to Bcl-2 and cytosol cyto C was increased, while mitochondrial cyto C was reduced. Cytosol cyto C can trigger the formation of the apoptosome containing apoptotic protease and activates caspases. Once activated, a single executioner caspase can cleave and activate other executioner caspases, leading to an accelerated feedback loop of caspase activation[24]. Caspase 9 is the first to be activated, and then activates procaspase 3, which carries out the process of apoptosis and is a sign of irreversible apoptosis.

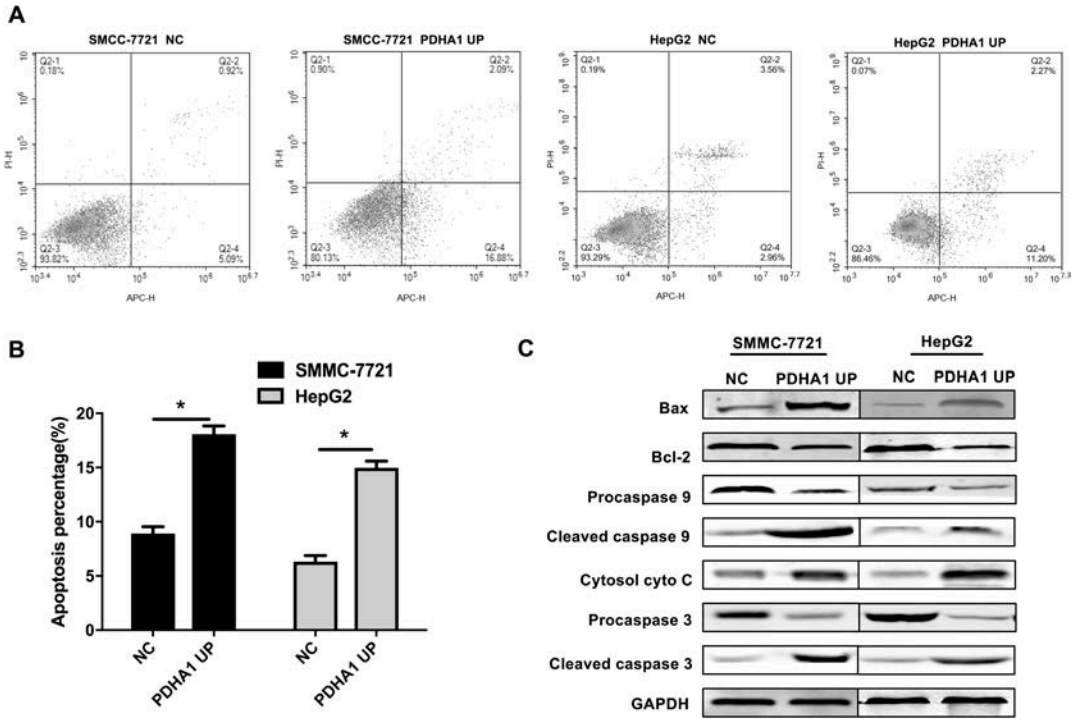

Figure 4. PDHA1 UP cells had a higher apoptosis rate through the mitochondrial pathway in SMMC-7721 and HepG2 cells. (A, B) The apoptosis of PDHA1 UP cells was significantly increased by flow cytometry. (C) Western blot analysis also shows higher expression levels of the mitochondrial apoptosis pathway-associated protein Bax, cleaved caspases 3 and 9, and cytosol cyto C in PDHA1 UP cells. The expression of Bcl-2 was decreased. The data are expressed as means ± SD of three independent experiments. *$p < 0.05$, compared to the NC group, according to the two-tailed Student's *t*-test. Abbreviation: cyto C, cytochrome C.

The test of related proteins of apoptotic pathways indicated that the expression of cleaved caspases 3 and 9 was significantly increased. All these results show that overexpression of the PDHA1 gene increased the apoptosis of HCC cells in vitro through the intrinsic apoptosis pathway.

Thus, we can conclude that overexpression of the PDHA1 gene can reduce glycolysis with lower lactate and increased OXPHOS with higher ATP generation through altering PDH activity. The increased apoptosis in PDHA1-overexpressing cells is induced via the mitochondrial pathway.

ACKNOWLEDGMENT: Zhixian Guo obtained the specimens. Lu Sun, Huijuan Cheng, Hongli Gu, Yan Yu, and Qiuyue Hu contributed to the study. Yubing Zhou provided language help. Quancheng Kan and Zujing Yu conducted the work. The authors declare no conflicts of interest.

REFERENCES

1. Warburg O. On respiratory impairment in cancer cells. Science 1956;124(3215):269–70.
2. Ma R, Zhang W, Tang K, Zhang H, Zhang Y, Li D, Li Y, Xu P, Luo S, Cai W, Ji T, Katirai F, Ye D, Huang B. Switch of glycolysis to gluconeogenesis by dexamethasone for treatment of hepatocarcinoma. Nat Commun. 2013;4:2508.
3. Kitamura K, Hatano E, Higashi T, Narita M, Seo S, Nakamoto Y, Yamanaka K, Nagata H, Taura K, Yasuchika K, Nitta T, Uemoto S. Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis. J Hepatol. 2011;55(4):846–57.
4. Beyoglu D, Imbeaud S, Maurhofer O, Bioulac-Sage P, Zucman-Rossi J, Dufour JF, Idle JR. Tissue metabolomics of hepatocellular carcinoma: Tumor energy metabolism and the role of transcriptomic classification. Hepatology 2013;58(1):229–38.
5. Beyoglu D, Idle JR. The metabolomic window into hepatobiliary disease. J Hepatol. 2013;59(4):842–58.
6. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4(11):891–9.
7. Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, Maguire C, Gammer TL, Mackey JR, Fulton D, Abdulkarim B, McMurtry MS, Petruk KC. Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med. 2010;2(31):31ra34.
8. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 2007;11(1):37–51.
9. Bianchi G, Martella R, Ravera S, Marini C, Capitanio S, Orengo A, Emionite L, Lavarello C, Amaro A, Petretto A, Pfeffer U, Sambuceti G, Sambuceti G, Pistoia V, Raffaqhello L, Longo VD. Fasting induces anti-Warburg effect that increases respiration but reduces ATP-synthesis to promote apoptosis in colon cancer models. Oncotarget 2015;6(14):11806–19.

10. Rajagopalan KN, Egnatchik RA, Calvaruso MA, Wasti AT, Padanad MS, Boroughs LK, Ko B, Hensley CT, Acar M, Hu Z, Jiang L, Pascual JM, Scaqlioni PP, DeBerardinis RJ. Metabolic plasticity maintains proliferation in pyruvate dehydrogenase deficient cells. Cancer Metab. 2015;3:7.
11. Patel MS, Korotchkina LG. Regulation of mammalian pyruvate dehydrogenase complex by phosphorylation: Complexity of multiple phosphorylation sites and kinases. Exp Mol Med. 2001;33(4):191–7.
12. Dupuy F, Tabaries S, Andrzejewski S, Dong Z, Blagih J, Annis MG, Omeroglu A, Gao D, Leung S, Amir E, Clemons M, Aquilar-Mahecha A, Basik M, Vincent EE, St-Pierre J, Jones RG, Sieqel PM. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metab. 2015;22(4):577–89.
13. Zhong Y, Huang R, Li X, Xu R, Zhou F, Wang J, Fan H, Goscinski M, Zhang M, Wen JG, Nesland JM, Suo Z. Decreased expression of PDHE1alpha predicts worse clinical outcome in esophageal squamous cell carcinoma. Anticancer Res. 2015;35(10):5533–8.
14. Li Y, Li X, Li X, Zhong Y, Ji Y, Yu D, Zhang M, Wen JG, Zhang H, Goscinski MA , Nesland JM, Suo Z. PDHA1 gene knockout in prostate cancer cells results in metabolic reprogramming towards greater glutamine dependence. Oncotarget 2016;7(33):53837–52.
15. Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D, Aldape K, Hunter T, Alfred Yung WK, Lu Z. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell 2012;150(4):685–96.
16. Cameron JM, Levandovskiy V, Mackay N, Tein I, Robinson BH. Deficiency of pyruvate dehydrogenase caused by novel and known mutations in the E1alpha subunit. Am J Med Genet A 2004;131(1):59–66.
17. Patel KP, O'Brien TW, Subramony SH, Shuster J, Stacpoole PW. The spectrum of pyruvate dehydrogenase complex deficiency: Clinical, biochemical and genetic features in 371 patients. Mol Genet Metab. 2012;106(3):385–94.
18. Menendez JA, Joven J, Cufi S, Corominas-Faja B, Oliveras-Ferraros C, Cuyas E, Martin-Castillo B, Lopez-Bonet E, Alarcon T, Vazquez-Martin A. The Warburg effect version 2.0: Metabolic reprogramming of cancer stem cells. Cell Cycle 2013;12(8):1166–79.
19. Garber K. Energy deregulation: Licensing tumors to grow. Science 2006;312(5777):1158–9.
20. Tyurina YY, Shrivastava I, Tyurin VA, Mao G, Dar HH, Watkins S, Epperly M, Bahar I, Shvedova AA, Pitt B, Wenzel SE, Mallampalli RK, Sadovsky Y, Gabrilovich D, Greenberqer JS, Bayir H, Kaqan VE. "Only a life lived for others is worth living": Redox signaling by oxygenated phospholipids in cell fate decisions. Antioxid Redox Signal. 2018;29(13):1333–58.
21. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 2009;324(5930):1029–33.
22. Antico Arciuch VG, Elguero ME, Poderoso JJ, Carreras MC. Mitochondrial regulation of cell cycle and proliferation. Antioxid Redox Signal 2012;16(10):1150–80.
23. Cory S, Adams JM. The Bcl2 family: Regulators of the cellular life-or-death switch. Nat Rev Cancer 2002;2(9): 647–56.
24. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol. 2015;7(4):a026716.