Int J Clin Exp Pathol 2017;10(11):10759-10769
www.ijcep.com /ISSN:1936-2625/IJCEP0061278

Original Article

Metabolic reprogramming induced by inhibition of SLC2A1 suppresses tumor progression in lung adenocarcinoma

Yanfen Wang[1*], Shanshan Shi[2*], Yongling Ding[1], Zheng Wang[1], Shuang Liu[1], Jiajia Yang[1], Tongpeng Xu[3]

[1]Department of Pathology, The Affliated Hospital of Yangzhou University, Yangzhou University, Yangzhou, Jiangsu, P. R. China; [2]Department of Pathology, Jinling Hospital, Clinical Medical School of Southern Medical University, Nanjing, Jiangsu, P. R. China; [3]Department of Oncology, The First Affliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, P. R. China. [*]Equal contributors.

Received July 11, 2017; Accepted August 1, 2017; Epub November 1, 2017; Published November 15, 2017

Abstract: Lung adenocarcinoma (LAC) is one of the common reasons of cancer-related death with few biomarkers for diagnosis and prognosis. Solute carrier family 2 member 1 protein, SLC2A1, has been associated with tumor progression, metastasis, and poor prognosis in many human solid tumors. However, little is reported about its biological functions in lung adenocarcinoma. Here we observed a strong up-regulation of SLC2A1 in patients with LAC and found that SLC2A1 was significantly correlated with prognosis. Knockdown of SLC2A1 in LAC cells inhibits cellular proliferation and plate clone formation *in vitro* as well as suppression of glucose utilization. Meanwhile, silencing of SLC2A1 also suppresses tumor metastasis *in vitro*. Mechanistically, GSEA showed that genes in cell cycle pathway were prominently enriched in the higher SLC2A1 group. By a large-scale proteomic analysis, we revealed that cell cycle protein level was significantly increased in SLC2A1-high group. Collectively, our findings indicate that elevated SLC2A1 is a critical modulator in lung adenocarcinoma progression by altering glucose metabolism and the cell cycle pathway, and also suggest SLC2A1 as a promising target for lung adenocarcinoma therapy.

Keywords: SLC2A1, tumor growth, invasion, glycometabolism, cell cycle, lung adenocarcinoma

Introduction

Lung cancer is one of the most malignant tumors and is the leading cause of cancer-related mortality worldwide [1]. Among lung cancers, lung adenocarcinoma (LAC) is the most common histopathological subtype, and despite recent advances in the elucidation of molecular mechanisms and surgical and chemotherapeutic interventions, the prognosis of LAC has not been improved satisfactory [2, 3]. Therefore, the molecular mechanisms of LAC need to be revealed in further detail to identify efficient molecular markers, including predictive markers for LAC, and to establish favorable treatment strategies for LAC. It has been reported that several regulators of the glycolysis pathway are detected in several premalignant lesions and tumors, which suggests that these proteins may participate in early carcinogenesis and progression of cancer [4]. Warburg effect not only promotes tumor cell proliferation by providing cellular building blocks, but also favors tumor cell metastasis by acidified microenvironment through increased production of lactate [5]. Therefore, glycolysis pathway might be a potential targetable pathway for cancer therapy.

Solute carrier family 2 facilitated glucose transporter member 1 (SLC2A1), also known as glucose transporter 1 (GLUT1), is a crucial protein in the cellular energy metabolism pathway. In normal tissues, GLUT1 is limited to be expressed on erythrocytes and endothelial cells in the blood-brain barriers [6]. Recently, SLC2A1 has been demonstrated to be a pivotal rate-limiting element in the transport of glucose in malignancy cells and over-expressed in several different types of human carcinomas, including

liver, pancreatic, endometrial, and breast cancers, as well as lung cancer [7-10]. These studies suggest that SLC2A1 could be one of the driver genes in tumors. However, the expression pattern and cellular functions of SLC2A1 in LAC remain largely unexplored.

In this study, we observed that SLC2A1 was over-expressed in LAC tumors by dataset analysis and found the significant correlation between SLC2A1 expression and prognosis. We further explored the functional significance of SLC2A1 in LAC tumorigenesis and demonstrated that SLC2A1 promoted LAC cells growth and enhanced LAC cells migration *in vitro* via enhancement of glucose utilization.

Material and methods

Cell culture and reagent

Human LAC cell lines NCI-H1793, NCI-H1975, NCI-H23 and HCC827 were all purchased from American Type Culture Collection (ATCC, Manassas, VA). All of these cells were cultured in indicated medium according to ATCC protocols, and supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics (100 μg/ml streptomycin and 100 units/ml penicillin) at 37°C in a humidified incubator under 5% CO_2 condition.

Quantitative real-time PCR

Total RNA was extracted from H1793 and HCC827 cells using Trizol reagent (Takara, Japan), and reversely transcribed through PrimeScript RT-PCR kit (Takara, Japan) according to the manufacturer's instructions. Quantitative real-time PCR was performed with SYBR Premix Ex Taq (Takara, Japan) on a 7500 Real-time PCR system (Applied Biosystems, Inc. USA). Primer sequences used in this study were as follows: SLC2A1, forward: 5'-AAGGTGATCGAGGAGTTCTACA-3', reverse: 5'-ATGCCCCCAACAGAA AAGATG-3'; β-actin, forward 5'-ACTCGTCATACTCCTGCT-3', reverse 5'-GAAACTACCTTCAACTCC-3'. The $2^{-\Delta Ct}$ method was used to quantify the relative SLC2A1 expression levels and normalized using the β-actin expression.

Data mining

The official report by the TCGA group on lung adenocarcinoma was published, and our reproduction abided by the rules of the TCGA request. Two types of data were downloaded from the TCGA website (https://cancergenome.nih.gov/): mRNA expression by RNA sequencing and protein level by reverse-phase protein arrays (PRPAs).

Western blotting

Cell total protein was extracted using a total protein extraction buffer (Beyotime, China) and the protein concentration was measured using a BCA Protein Assay Kit (Pierce Biotechnology). Cell lysates were separated by 8-12% SDS-PAGE gel electrophoresis and transferred to a PVDF membrane. After blocking with 1% BSA, the membrane was probed with one of the following primary antibodies: SLC2A1, β-actin, CCNB1, CCND1 (Proteintech Group, US) and species-specific secondary antibodies (CST, US). Bound secondary antibodies were detected by Odyssey imaging system (LI-COR Biosciences, Lincoln, NE).

Establishment of stable SLC2A1 knockdown cell lines

Short hairpin RNA (shRNA)-containing plasmids were packaged into lenti-virus and virus titers were determined. Two target cell lines, H1793 and HCC827, were infected with 1×10^6 recombinant lentivirus-transducing units in the presence of 6 μg/ml polybrene (Sigma, Shanghai, China). The sequences targeting SLC2A1 are as follows; sh-1: 5'-CCGGGCGGAATTCAATGC TGATGATCTCGAGATCATCAGCATTGAATTCCGCTTTTTG-3'; sh-2: 5'-CCGGCTTCTATTACTCCACGAGCATCTCGAGATGCTCGTGGAGTAATAGAAGTTTTTG-3'. The stable SLC2A1 knockdown cells were selected in the presence of 2 μg/ml puromycin. The knockdown efficacy was tested by western blotting and real time PCR.

Cell viability assay

For cell viability assay, cells were seeded into a 96-well plate at 3×10^3 cells per well with 100 μl culture medium and cultured at 37°C. The cell viability was quantified by Cell Counting Kit-8 (CCK-8, Dojindo, Japan). Briefly, by addition of 10% (v/v) CCK-8 to the culture medium and incubation for 1.5 h, cell viability was monitored by measuring absorbance at 450 nm using a Power Wave XS microplate reader (BIOTEK). The experiment was performed in quintuplicate and repeated twice.

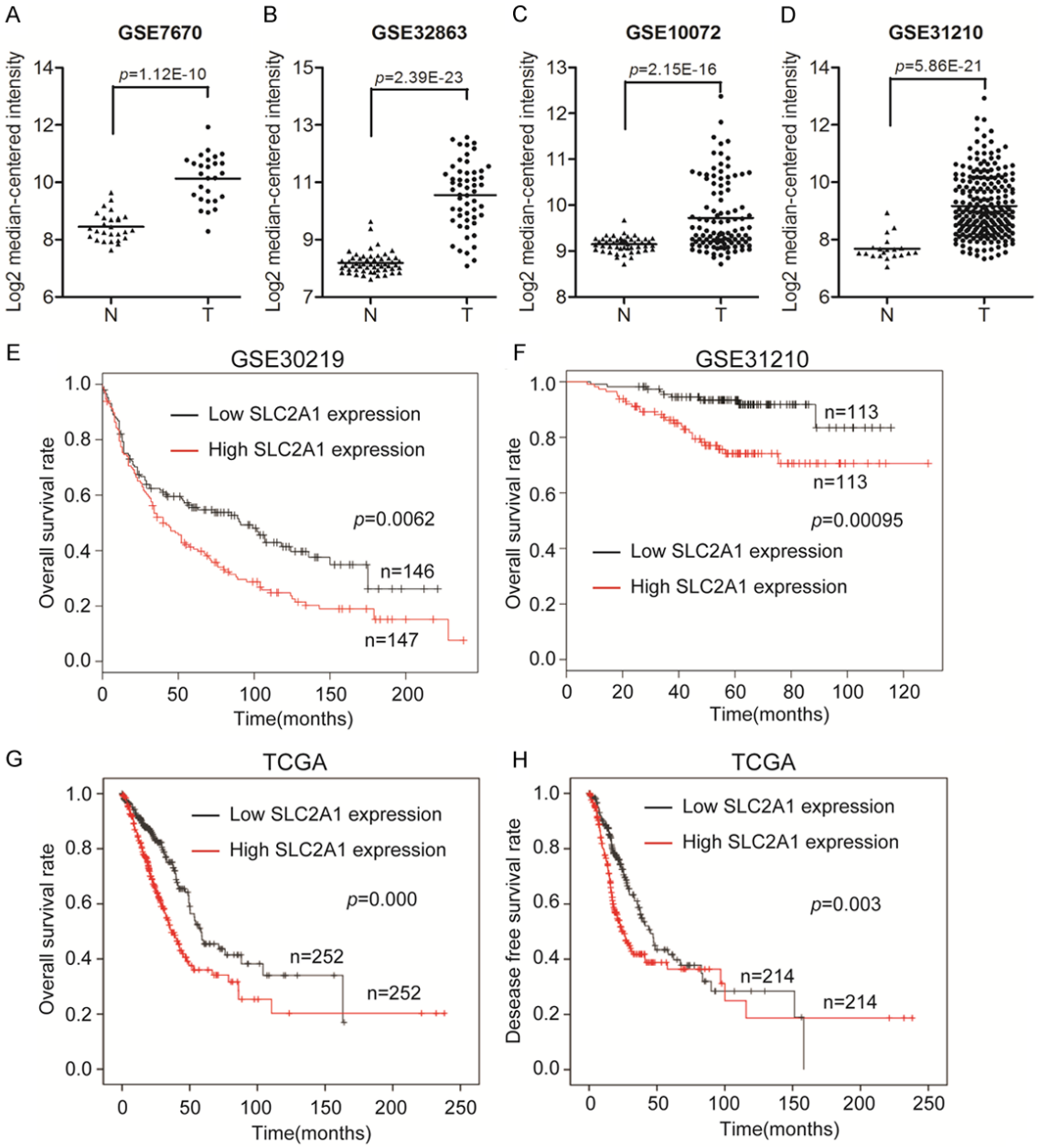

Figure 1. SLC2A1 expression is increased in lung adenocarcinama and correlated with survival rate. (A-D) The mRNA expression of SLC2A1 is upregulated in LAC tissues (T) compared with the normal lung tissues (N) revealed using the GSE7670 dataset (A), GSE3283 dataset (B), GSE10072 (C) and GSE31210 dataset (D). (E, F) The correlation between SLC2A1 expression and patient survival was conducted in GSE30219 dataset (E) and GSE31210 dataset (F). (G, H) Comparisons of overall survival (G) and disease-free survival (H) between lower SLC2A1 expression group and higher SLC2A1 expression group in TCGA data. *P* value was calculated by log-rank test.

Cell invasion assay

The invasive ability of LAC cells was measured by transwell model (Corning, NY, USA) according to the manufacturer's instructions. 700 μl RPMI 1640 or DMEM medium containing 5% (v/v) FBS was added to the lower chambers. 2 × 10^4 Cells in 100 μl 1640 or DMEM medium were seeded into the upper compartment coated with 100 μL matrigel (BD Bioscience, USA). After the cells were incubated for 48 h, the non-invading cells remained on the upper surface were scraped off. The invaded cells were fixed with 4% paraformaldehyde and stained with

Table 1. Relationship between SLC2A1 expression and clinicopathological features of LAC patients

	Total	SLC2A1				*P* Value
		Low	%	High	%	
Age (years)						
< 60	136	57	41.9	79	58.1	0.015*
≥ 60	362	193	53.3	169	46.7	
Gender						
Male	239	105	43.9	134	56.1	0.006**
Female	278	154	55.4	124	44.6	
Tumor size						
≤ 3 cm	171	104	60.8	67	39.2	0.005**
> 3 cm	277	130	46.9	147	53.1	
Stage						
I	281	158	56.2	123	43.8	0.018*
II	124	54	43.5	70	56.5	
III	84	34	40.5	50	59.5	
IV	26	11	42.3	15	57.7	
Lymph node metastasis						
Absent	334	179	53.6	155	46.4	0.011*
Present	171	71	41.5	100	58.5	
Distant metastasis						
No	348	177	50.9	171	49.1	0.407
Yes	24	10	41.7	14	58.3	

*, $P < 0.05$, **, $P < 0.01$.

0.1% crystal violet. The number of cells on the lower surface was counted under a light microscope in six random fields. Each experiment was performed in triplicate and repeated twice.

Measurement of pyruvate, lactate and glucose

Cells were cultured in 24 well plates for 24 h. Pyruvate and lactate production was measured by the enzymetic method using a commercially available luorescence-based assay kit (Abcam, US). The glucose in the conditioned media was quantified using the Glucose Assay kit (Sigma, Shanghai, China). Quantities of glucose consumed were normalized to the DNA content of each well and triplicate samples were analyzed.

3H-2-deoxyglucose transport assay

Cells were incubated in glucose-free Dulbecco's modified eagle's medium (DMEM) and then pulsed with 2 μCi of 3H-2-deoxyglucose (~60 pmol) (PerkinElmer, Nanjing, China) for 10 min. The monolayers were quantitated by liquid scintillation counting. The data is presented as counts per minute (CPM) per μg DNA.

Statistical analysis

Data were presented as the means ± SD. The SPSS software program (version 19.0; IBM Corporation) was used for statistical analysis. Graphical representations were performed with GraphPad Prism 5 (San Diego, CA) software. Survival curves were evaluated using the Kaplan-Meier method and differences between survival curves were tested by the log-rank test. The student's *t*-test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

Results

SLC2A1 is over-expressed in LAC tissues and predicts poor prognosis

To illustrate the expression pattern of SLC2A1 in LAC, we searched the mRNA expression level of SLC2A1 in four GEO datasets. The results showed that SLC2A1 expression was significantly up-regulated in LAC tissues comparing with paired normal pancreatic tissues using GSE7670 (**Figure 1A**, $n = 27$, $P = 1.12E-10$) and GSE32863 (**Figure 1B**, $n = 54$, $P = 2.39E-23$). Expression of SLC2A1 was also remarkably higher in the LAC tissues than the unpaired normal lung tissues as revealed by GSE10072 (**Figure 1C**, $P = 2.15E-16$) and GSE31210 (**Figure 1D**, $P = 5.86E-21$).

To evaluate the prognostic significance of SLC2A1 in LAC patients, the correlation between SLC2A1 expression and corresponding clinical follow-up information were analyzed using Kaplan-Meier method. We determined the prognostic value of SLC2A1 at mRNA level using two GEO datasets and TCGA RNAseq dataset. As shown in **Figure 1E** and **1F**, in GEO datasets, patients with higher SLC2A1 level had significantly shorter survival time than those with a lower SLC2A1 level. Similarly, in TCGA dataset, patients with higher SLC2A1 level had marked shorter overall survival time and disease free survival time (**Figure 1G** and

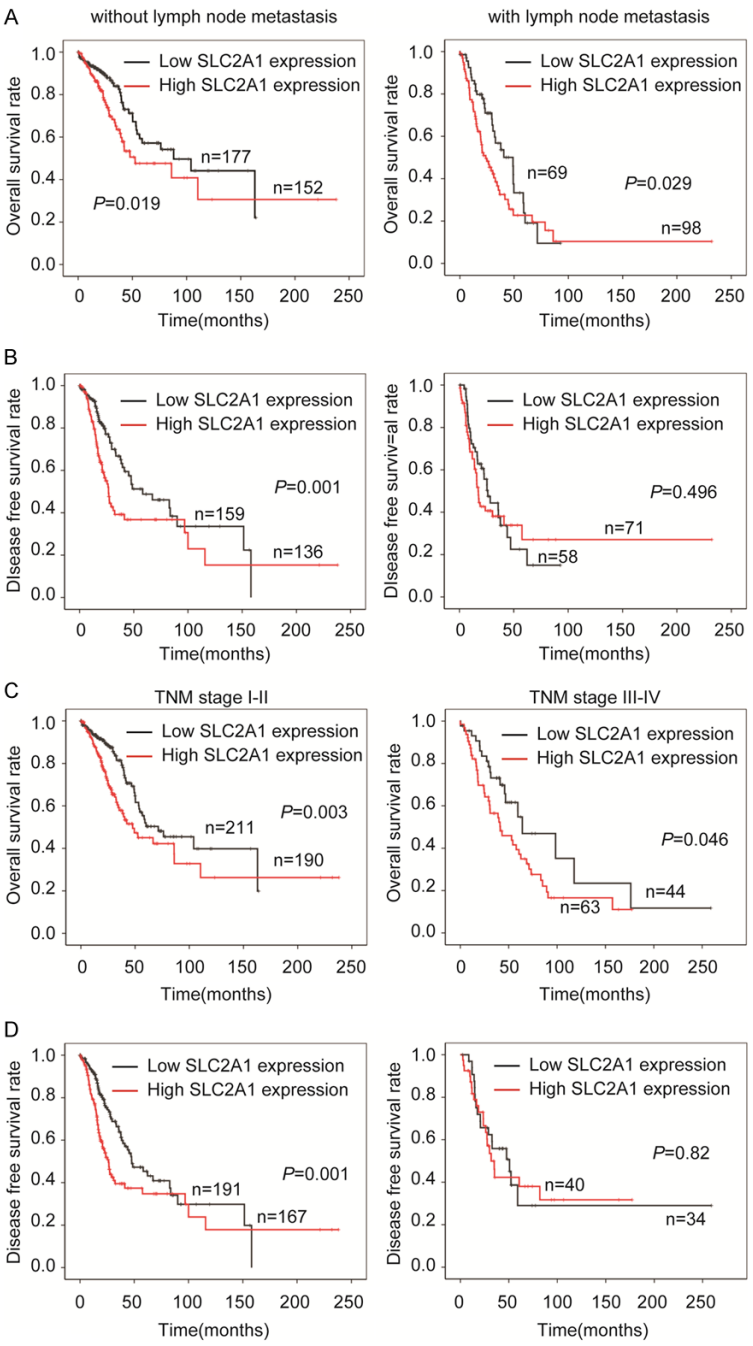

Figure 2. SLC2A1 expression is correlated with disease-free survival rate dependent on TNM stage and lymph node metastasis. A: Comparison of overall survival in patients with or without lymph node metastasis was conducted based on SLC2A1 expression. B: Comparison of disease-free survival in patients with or without lymph node metastasis was conducted based on SLC2A1 expression. C: Comparisons of overall survival between lower SLC2A1 expression group and higher SLC2A1 expression group in early TNM stage (I-II) cohort and in advanced TNM stage (III-IV) cohort. D: Comparisons of disease-free survival between lower SLC2A1 expression group and higher SLC2A1 expression group in early TNM stage (I-II) cohort and in advanced TNM stage (III-IV) cohort. *P* value was calculated by log-rank test.

1H). Taken together, these data above suggest that up-regulated SLC2A1 predicts poor prognosis and might contribute to tumor progression in LAC patients.

Relationship between SLC2A1 expression and clinical parameters in patients with LAC

To determine the clinical significance of SLC2A1 expression in LAC, the Chi-square test was used to assess the relationships between SLC2A1 Mrna expression and corresponding patients' clinicopathologic parameters including age, gender, TNM stage, tumor size, lymph node metastasis and distant metastasis in TCGA data. The results showed that SLC2A1 mRNA expression in LAC tissues was significantly correlated with age (P = 0.015), gender (P = 0.006), TNM stage (P = 0.018), tumor size (P = 0.005), lymph node metastasis (P = 0.011), while no significant associations were observed between SLC2A1 expression and distant metastasis (**Table 1**).

Correlations between SLC2A1 mRNA expression and prognosis of LAC patients

We further evaluated the correlation between SLC2A1 expression and overall survival rate or disease-free survival rate of LAC patients in early or advanced TNM stage and in the presence or absence of lymphatic metastasis. Kaplan-Meier analyses showed that overall survival time was shorter in LAC patients with higher SLC2A1 expression regardless the state of lymphatic metastasis (**Figure 2A**) and TNM stage (**Figure 2C**). While

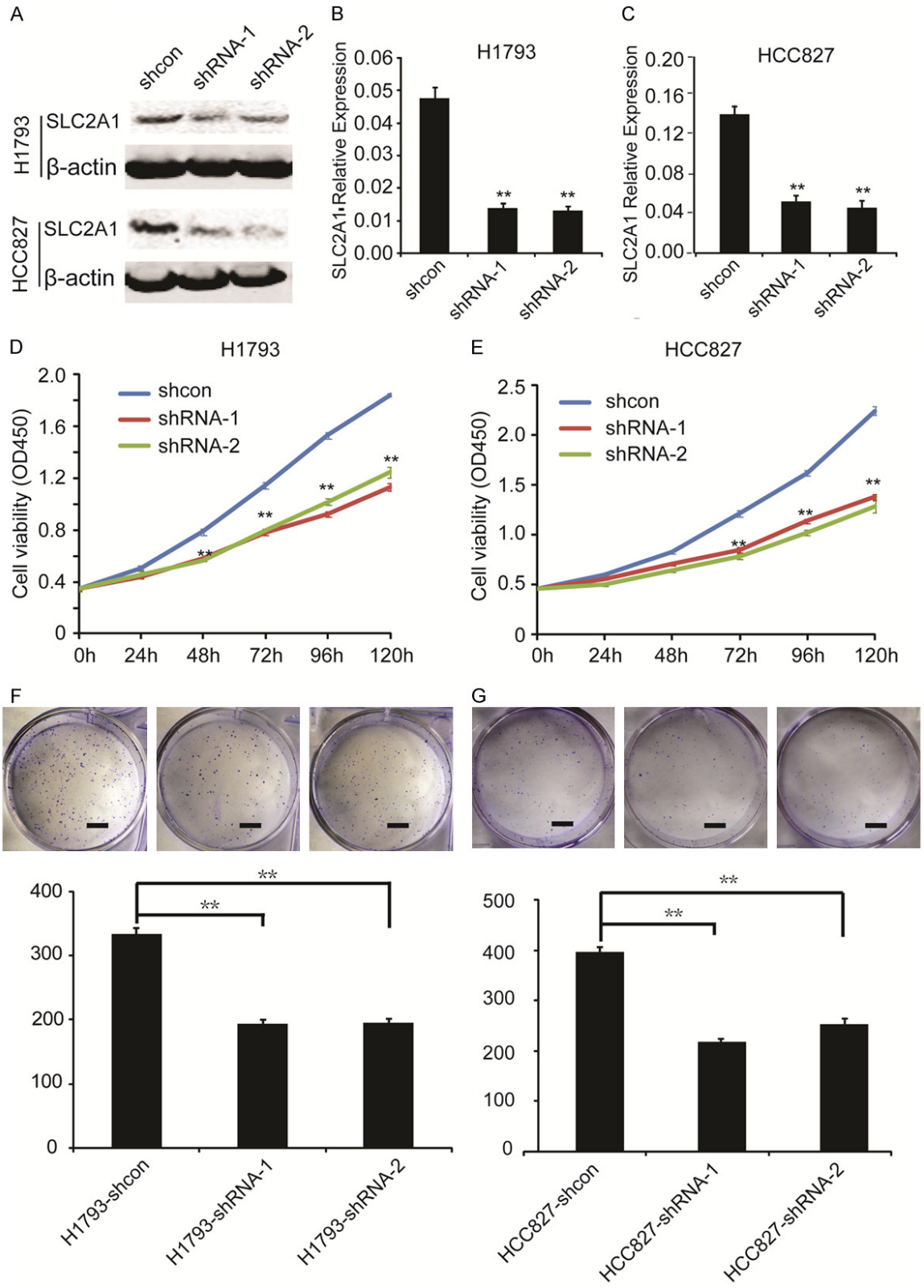

Figure 3. Knockdown of SLC2A1 inhibited LAC cells viability *in vitro*. Interfere efficacy in H1793 and HCC827 cells was detected by Western blotting (A) and quantitative real-time PCR (B and C). Cell counting kit-8 (CCK-8) assay showed silencing of SLC2A1 inhibited cell growth in H1793 (D) and HCC827 cells (E). Knockdown of SLC2A1 inhibited the colony formation ability of H1793 (F) and HCC827 (G) cells, Scale bar: 5 mm. shcon versus shRNA-1 or shRNA-2, $^{**}P < 0.01$.

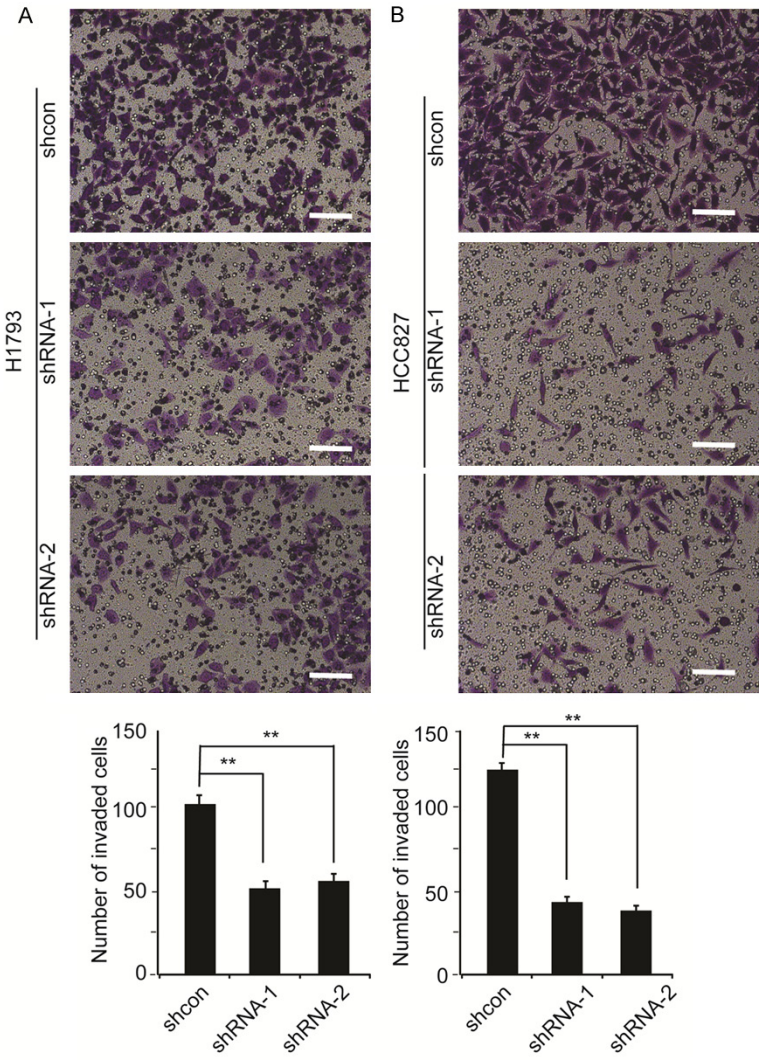

Figure 4. Knockdown of SLC2A1 inhibited LAC cells invasion *in vitro*. The invasive potential of SLC2A1-silencing H1793 (A) and HCC827 cells (B) was assessed by Transwell model. Scale bar: 50 μm, shcon versus shRNA-1 or shRNA-2, **, $P < 0.01$.

the correlation between SLC2A1 expression and disease-free survival rate of LAC patients was dependent on lymphatic metastasis (**Figure 2B**) and TNM stage (**Figure 2D**). In the absence of lymphatic metastasis and early TNM stage, disease-free survival time was also shorter in LAC patients with higher SLC2A1 expression. In advanced TNM stage and the presence of lymphatic metastasis, disease-free survival rate of LAC patients had no significant associations with SLC2A1 level.

Suppression of SLC2A1 inhibits LAC cells growth in vitro

We established stable cell lines expressing short hairpin RNA targeting SLC2A1 in H1793 and HCC827 cells. Knockdown efficiency of SLC2A1 in the two cell lines was verified by western blot and real time PCR. As shown in **Figure 3A-C**, Stable expression of two short hairpin RNA (shRNA-1, shRNA-2) resulted in > 75% decrease in SLC2A1 expression. Cell counting kit-8 (CCK-8) assay showed that knockdown of SLC2A1 expression induced significant decreases in H1793 cellular viability (**Figure 3D**). Similarly, the HCC827 cells suppressed SLC2A1 expression grew slower than the control (**Figure 3E**). To further validate the role of SLC2A1 in cancer cell survival, clonogenic assay was performed. As shown in **Figure 3F** and **3G**, suppression of SLC2A1 significantly reduced the colony formation of H1793 and HCC827 cells.

Silencing of SLC2A1 inhibits LAC cells invasion in vitro

By Transwell model, we investigated the effect of SLC2A1 on LAC cells invasion. We observed that fewer numbers of invaded cells in shRNA group compared with the control group in both H1793 ($P < 0.01$, **Figure 4A**) and HCC827 ($P < 0.01$, **Figure 4B**) cells. These data suggest that knockdown of SLC2A1 provides a disadvantage for LAC cell metastasis *in vitro*.

Knockdown of SLC2A1 depresses LAC cells glucose utilization in vitro

Next, we sought to determine whether the LAC cells with decreased SLC2A1 protein had altered glucose utilization. As shown in **Figure 5A**, SLC2A1-shRNA HCC827 cells consumed fewer glucose than the control ($P < 0.01$). Similarly, HCC827 cells silencing of SLC2A1 took fewer 3H-2-deoxyglucose (3H-2-DOG) compared to the control cells (**Figure 5B**, $P < 0.01$). Meanwhile, we found a decrease in fructose-6-phosphate (**Figure 5C**, $P < 0.001$) and

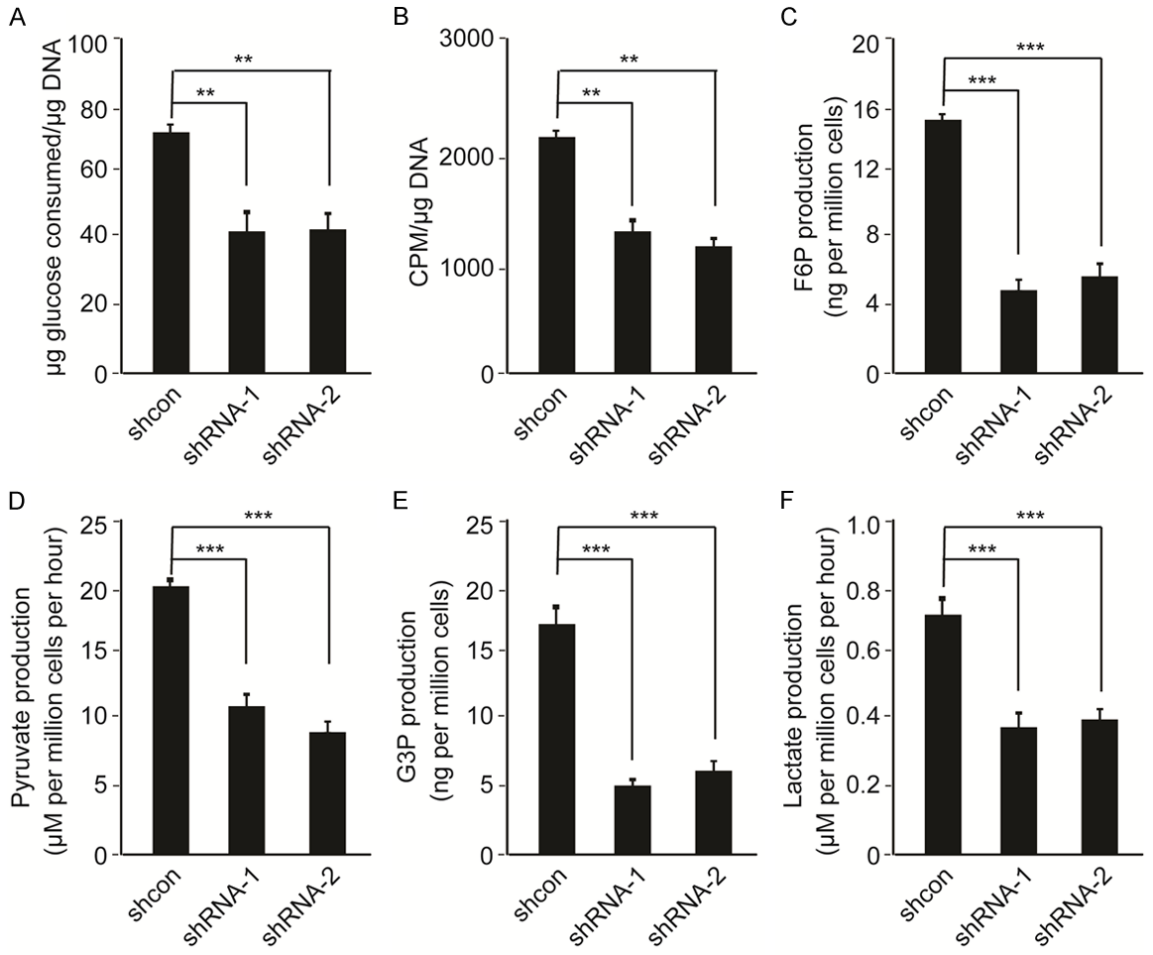

Figure 5. Knockdown of SLC2A1 inhibited glucose utilization of LAC cells. A: SLC2A1-silencing HCC827 cells consumed fewer glucose than controls. B: SLC2A1- silencing HCC827 cells took fewer 3H-2-deoxyglucose (3H-2-DOG) compared with the control cells. C: The quantitative changes of fructose-6-phosphate (F6P) in SLC2A1- silencing HCC827 cells compared with that in control cells. D: The production level of pyruvate in the culture buffer of SLC2A1- silencing HCC827 cells and control cells. E: The quantitative changes of G3P in SLC2A1-silencing HCC827 cells compared with that in control cells. F: The production level of lactate in the culture buffer in SLC2A1- silencing HCC827 cells and control cells. shcon versus shRNA-1 or shRNA-2, **, $P < 0.01$, ***, $P < 0.001$.

pyruvate production (**Figure 5D**, $P < 0.001$) in SLC2A1-slilencing HCC827 cells compared with vehicle cells. Furthermore, we found significantly decreased glyceraldehydes-3-phosphate production (G3P) in SLC2A1- silencing HCC827 cells compared with vehicle cells (**Figure 5E**, $P < 0.001$). Similarly, there's a decrease in lactate production in SLC2A1- silencing HCC827 cells compared with vehicle cells (**Figure 5F**, $P < 0.001$). These data demonstrate that knockdown of SLC2A1 expression leads to a deraction of glucose transport, glucose consumption and lactate secretion, indicating the critical roles of SLC2A1 in glycolysis and glucose metabolism. Overall, these data support that LAC cells with increased SLC2A1 protein had enhanced glucose utilization.

SLC2A1 correlates increased cell cycle protein level in lung adenocarcinoma

To illustrate the mechanism by which SLC2A1 promotes tumor progression in lung adenocarcinoma, Gene Set Enrichment Analysis (GSEA) was performed in the expression profiles of LAC tissues in TCGA. Based on SLC2A1 expression level in TCGA dataset, top fifty tissues were defined higher group and posterior fifty tissues were defined lower group. The difference of SLC2A1 expression between two groups was significant (**Figure 6A**). GSEA showed that genes in cell cycle pathway were prominently enriched in the higher SLC2A1 group, indicating that SLC2A1 is a potential promoter of cell cycle (**Figure 6B**). To test this,

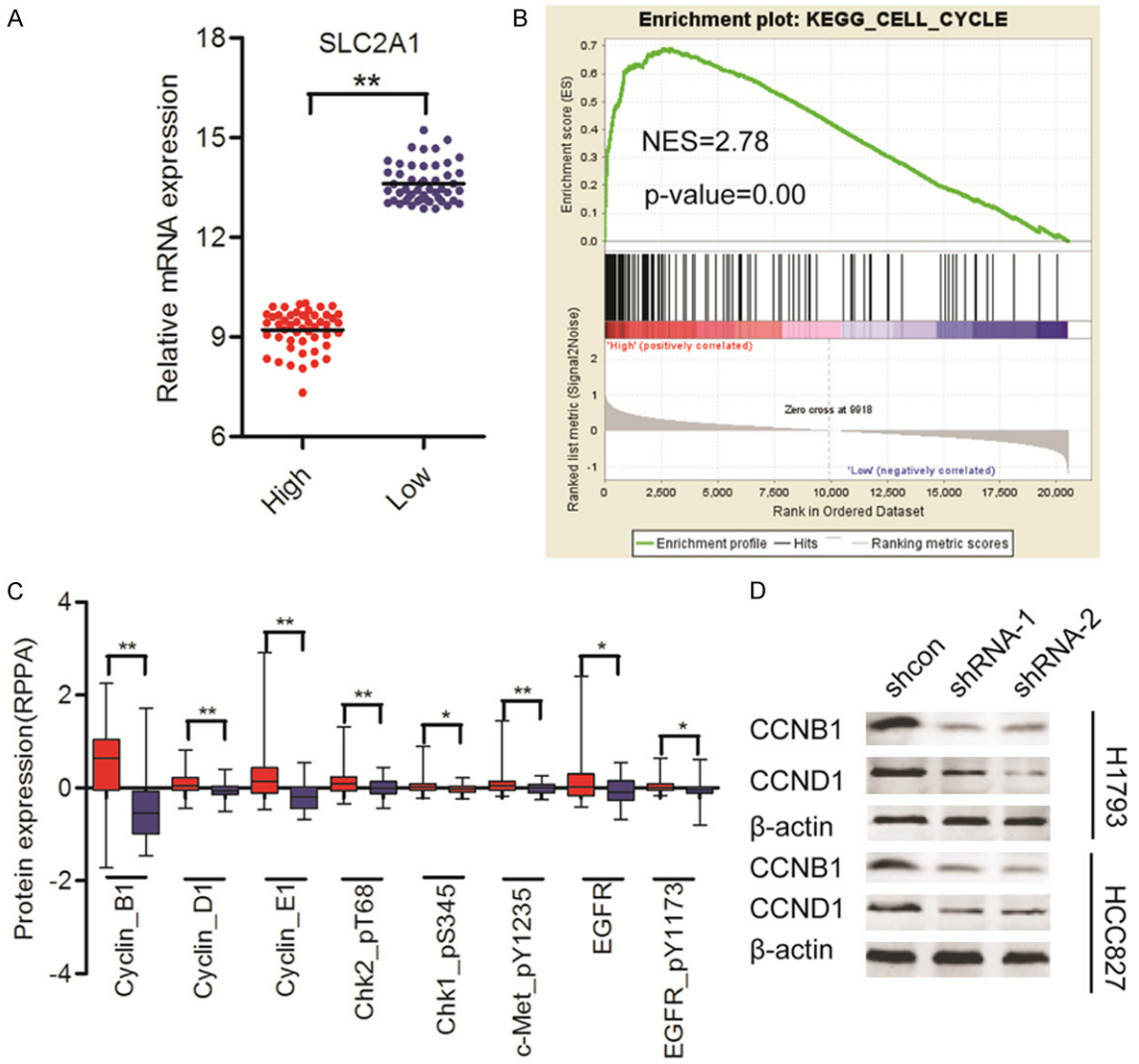

Figure 6. SLC2A1 is positive regulator of cell cycle in lung adenocarcinoma. A: The expression level of SLC2A1 in lower group (n = 50) and high group (n = 50). B: Genes in cell cycle pathway identified by GSEA were enriched in SLC2A1 high group. C: Differentially expressed proteins between SLC2A1-high and SLC2A1-low group in TCGA. D: The CCNB1 and CCND1 protein expression in H1793 and HCC827 cells. *, $P < 0.05$; **, $P < 0.01$.

we analyzed the levels of cancer-related total and phosphorylated proteins using the reverse-phase protein arrays (PRPAs) located in the TCGA dataset. The result showed that cyclin B1, cyclin D1, cyclin E1, CHK2_pT68, CHK1_pS345, MET-pY1235, EGFR and EGFR_pY1173 was significantly higher in SLC2A1-high group (**Figure 6C**). In addition, knockdown of SLC2A1 expression induced significant decreases in Cyclin B1 and Cyclin D1 protein expression in H1793 and HCC827 cells (**Figure 6D**). Taken together, these results indicate that the oncogenic functions of SLC2A1 in lung adenocarcinoma may associate with cell cycle.

Discussion

SLC2A1 gene encodes GLUT1 protein and is one of the isoforms of the protein family of facilitative glucose transporters, which is highly concentrated in tissue endothelium and epithelium [11]. Considering glucose uptake is the first rate-limiting step in aerobic glycolysis, SLC2A1 is often found to be over-expressed in various cancer types, including oral, liver, lung, breast, and endometrial cancer, due to the higher demand of glucose [7, 9, 10, 12]. In lung cancer, SLC2A1 expression is upregulated at both the RNA and protein levels in premalig-

nant lesions of lung squamous cell carcinoma, when compared with normal lung epithelial basal cells from the same patients [13]. In lung adenocarcinoma, two previous studies showed that SLC2A1 could be a prognostic marker [14, 15]. However, its underlying biological functions in LAC cells remains unknown.

In our study, by systematic analysis of several datasets from GEO and TCGA, we also observed that SLC2A1 level was commonly up-regulated in clinical specimens compared with the normal lung tissues. To address the prognostic value of SLC2A1 in LAC, we performed Kaplan-Meier survival analyses. Consistent with previous studies, our results indicated that SLC2A1 expression significantly correlated with patients' overall survival and disease freesurvival. Elevated expression of SLC2A1 was inversely associated with clinical outcomes of LAC patients.

Up-regulated SLC2A1 expression contributes to carcinogenesis and tumor progression. In this study, we analyzed the oncogenic activity of SLC2A1 in LAC cell lines. We established stable cell lines silencing SLC2A1 in H1793 and HCC827 cells to further explore the function of SLC2A1. Firstly, we observed that knockdown of SLC2A1 significantly inhibited LAC cell viability and colony formation. Secondly, silencing of SLC2A1 markedly suppressed LAC cells invasion. Finally, SLC2A1-silencing HCC827 cells took fewer 3H-2-DOG, consumed fewer glucose and produced fewer fructose-6-phosphate, glyceraldehydes-3-phosphate, pyruvate and lactate compared with control. These results consistent with that in gastric cancer. Consistent with this observation, it was reported that overexpression of SLC2A1 promotes tumor cell proliferation and metastasis in gastric cancer by promoting glucose utilization [16]. And in disease progression from benign tissue to prostate cancer, glyceraldehydes-3-phosphate production was also increased [17]. Given the roles of SLC2A1 in enhancing glucose utilization, thus supporting energy requirements, providing enormous biosynthetic needs and promoting the secretion of lactate and further contributing to the acidification of tumor microenvironment, which ultimately favors tumor growth and progression, it is reasonable to expect the oncogenic functions in lung adenocarcinama.

In conclusion, we find that elevated SLC2A1 expression is significantly correlated with LAC patients' prognosis. Meanwhile, knockdown of SLC2A1 inhibits the proliferation, colony formation, invasion and glucose utilization of LAC cells. These results suggest that SLC2A1 might acts as a candidate target for developing treatment of LAC.

Disclosure of conflict of interest

None.

Address correspondence to: Tongpeng Xu, Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu, P. R. China. E-mail: tpengxu@sina.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Hung JJ, Yeh YC, Jeng WJ, Wu KJ, Huang BS, Wu YC, Chou TY and Hsu WH. Predictive value of the international association for the study of lung cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma in tumor recurrence and patient survival. J Clin Oncol 2014; 32: 2357-2364.

[3] Devarakonda S, Morgensztern D and Govindan R. Genomic alterations in lung adenocarcinoma. Lancet Oncol 2015; 16: e342-351.

[4] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[5] Asgari Y, Zabihinpour Z, Salehzadeh-Yazdi A, Schreiber F and Masoudi-Nejad A. Alterations in cancer cell metabolism: the Warburg effect and metabolic adaptation. Genomics 2015; 105: 275-281.

[6] Pardridge WM, Boado RJ and Farrell CR. Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. J Biol Chem 1990; 265: 18035-18040.

[7] Amann T, Maegdefrau U, Hartmann A, Agaimy A, Marienhagen J, Weiss TS, Stoeltzing O, Warnecke C, Scholmerich J, Oefner PJ, Kreutz M, Bosserhoff AK and Hellerbrand C. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. Am J Pathol 2009; 174: 1544-1552.

[8] Higashi T, Tamaki N, Torizuka T, Nakamoto Y, Sakahara H, Kimura T, Honda T, Inokuma T, Katsushima S, Ohshio G, Imamura M and Konishi J. FDG uptake, GLUT-1 glucose transporter and cellularity in human pancreatic tumors. J Nucl Med 1998; 39: 1727-1735.

[9] Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A and Brys M. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. Pathol Oncol Res 2012; 18: 721-728.

[10] Wachi S, Yoneda K and Wu R. Interactome-transcriptome analysis reveals the high centrality of genes differentially expressed in lung cancer tissues. Bioinformatics 2005; 21: 4205-4208.

[11] Manel N, Kim FJ, Kinet S, Taylor N, Sitbon M and Battini JL. The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. Cell 2003; 115: 449-459.

[12] Kunkel M, Reichert TE, Benz P, Lehr HA, Jeong JH, Wieand S, Bartenstein P, Wagner W and Whiteside TL. Overexpression of Glut-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma. Cancer 2003; 97: 1015-1024.

[13] Ooi AT, Gower AC, Zhang KX, Vick JL, Hong L, Nagao B, Wallace WD, Elashoff DA, Walser TC, Dubinett SM, Pellegrini M, Lenburg ME, Spira A and Gomperts BN. Molecular profiling of premalignant lesions in lung squamous cell carcinomas identifies mechanisms involved in stepwise carcinogenesis. Cancer Prev Res (Phila) 2014; 7: 487-495.

[14] Maki Y, Soh J, Ichimura K, Shien K, Furukawa M, Muraoka T, Tanaka N, Ueno T, Yamamoto H, Asano H, Tsukuda K, Toyooka S and Miyoshi S. Impact of GLUT1 and Ki-67 expression on earlystage lung adenocarcinoma diagnosed according to a new international multidisciplinary classification. Oncol Rep 2013; 29: 133-140.

[15] Stewart PA, Parapatics K, Welsh EA, Muller AC, Cao H, Fang B, Koomen JM, Eschrich SA, Bennett KL and Haura EB. A pilot proteogenomic study with data integration identifies MCT1 and GLUT1 as prognostic markers in lung adenocarcinoma. PLoS One 2015; 10: e0142162.

[16] Yan S, Wang Y, Chen M, Li G and Fan J. Deregulated SLC2A1 Promotes Tumor Cell Proliferation and Metastasis in Gastric Cancer. Int J Mol Sci 2015; 16: 16144-16157.

[17] Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C and Chinnaiyan AM. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 2009; 457: 910-914.