Biochemical and Biophysical Research Communications 463 (2015) 440–446

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The regulation of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase by autophagy in low-glycolytic hepatocellular carcinoma cells

Jeong Yong Jeon [a, 1], Hyangkyu Lee [b, 1], Jeayeo Park [b], Misu Lee [a], Sae Whan Park [a], Ji Sook Kim [a], Milim Lee [a], Byoungchul Cho [c], Kyungsup Kim [d], Augustine M.K. Choi [e], Chun K. Kim [f, **], Mijin Yun [a, *]

[a] Department of Nuclear Medicine, Severance Hospital, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[b] Department of Clinical Nursing Science, Yonsei University College of Nursing, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[c] Division of Medical Oncology, Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[d] Department of Biochemistry and Molecular Biology, Brain Korea 21 Project for Medical Science, Institute of Genetic Science, Integrated Genomic Research Center for Metabolic Regulation, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[e] Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medical College and New York-Presbyterian Hospital, New York, NY, USA
[f] Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02026, USA

ARTICLE INFO

Article history:
Received 12 May 2015
Accepted 29 May 2015
Available online 30 May 2015

Keywords:
Gluconeogenesis
Autophagy
Hepatocellular carcinoma
Starvation
Glycolysis

ABSTRACT

The glycolytic phenotype is a dominant metabolic phenomenon in cancer and is reflected in becoming aggressive. Certain hepatocellular carcinoma lack increased glycolysis and prefer to uptake acetate than glucose for metabolism. Autophagy plays a role in preserving energies and nutrients when there is limited external nutrient supply and maintains glucose level of blood though supporting gluconeogenesis in the liver. As the role of autophagy and gluconeogenesis in HCC following the glycolic activity was not clear, we cultured HCC cells with different glycolytic levels in Hank's balanced salt solution (HBSS) to induce autophagy and conducted the activity of gluconeogenesis. Both autophagy and gluconeogenesis were induced in low glycolytic HCC cells (HepG2). In glycolytic Hep3B cells, only autophagy without gluconeogenesis was induced upon starvation. When autophagy was blocked, the level of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) was reduced in HepG2 cells and not in Hep3B. Altogether, we investigated contribution of hepatic gluconeogenesis to the metabolic phenotype of HCC cells and the role of autophagy as a potential mechanism regulating gluconeogenesis in low glycolytic HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is an incurable type of cancer with limited treatment options in advanced stages. Although recent developments in the underlying pathogenesis of HCC have introduced new molecular targets and novel therapeutic agents, patient survival in cases of advanced disease has not improved [1,2].

The glycolytic phenotype is a dominant metabolic phenomenon in cancer. Glycolytic tumors with increased ^{18}F-FDG uptake use glucose as a nutrient source for proliferation, whereas low glycolytic tumors show increased ^{11}C-acetate uptake accompanying lipid synthesis [3]. This intriguing pattern of nutrient preference has

Abbreviations: AMPK, AMP-activated protein kinase; CQ, chloroquine; FDG, fluorodeoxyglucose; G6Pase, glucose-6-phosphatase; HBSS, Hank's balanced salt solution; HCC, hepatocellular carcinoma; LC3B, microtubule-associated protein 1 light chain 3 beta; mTOR, mammalian target of rapamycin; p62 (SQSTM1), sequestosome 1; PRPCK, phosphoenolpyruvate carboxykinase.

* Corresponding author.
** Corresponding author. Division of Nuclear Medicine and Molecular Imaging, Brigham and Women's Hospital, 75 Francis St, Boston, MA 02115, USA.
E-mail addresses: ckkim@bwh.harvard.edu (C.K. Kim), yunmijin@yuhs.ac (M. Yun).

[1] Equal contributors.

http://dx.doi.org/10.1016/j.bbrc.2015.05.103
0006-291X/

been well correlated to histological grades of HCCs, with glycolytic cancers having a higher histological grade [4]. Despite the extensive use of cancer metabolism to grade the biological aggressiveness of HCCs and predict patient prognosis [5,6], underlying molecular mechanisms of the low glycolytic phenotype of HCCs are not well studied.

In high-grade HCCs and most malignant tumors, increased glucose is transported into the cytoplasm via glucose transporters and is phosphorylated by hexokinase, forming G-6-phosphate. Glucose-6-phosphate enters the glycolytic pathway in the cells. In contrast, the amount of uptake glucose decreases, likely due to high glucose-6-phosphatase (G6Pase) activity in low-grade HCCs [7,8]. Since one of the most important functions of the liver is the production of glucose to maintain blood glucose levels during fasting, high G6Pase activity was attributed to the preservation of the normal metabolic functions of the liver, such as gluconeogenesis in low-grade HCCs [9]. However, it has not yet been proved whether gluconeogenesis is one of the mechanisms for some low-grade HCCs using acetate as main metabolic source.

Substrates must be supplied to ensure the production of new glucose in the process of hepatic gluconeogenesis. Autophagy is a process of metabolic compensation in cells under fasting or unfavorable cell conditions [10]. The process forms specialized cytoplasmic vesicles called autophagosomes that encircle the damaged intracellular organelles and macromolecules. Those autophagosomes eventually fuse with lysosomes for the degradation of enclosed contents, thus recycling the sugars, fatty acids, and amino acids into the cytoplasm [11]. In liver, autophagy has been reported to be an important mechanism for supplying amino acid substrates from autophagic proteolysis for gluconeogenesis to maintain blood glucose levels in a fasting animal model [12,13].

In this study, the contribution of hepatic gluconeogenesis to the metabolic phenotype of HCC and the role of autophagy as a potential mechanism regulating gluconeogenesis were studied in HCC cell lines with different glycolytic phenotype.

2. Materials and methods

2.1. Cell lines and culture

Human HCC cell lines (HepG2 and Hep3B) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were maintained in Dulbecco's modified Eagle medium (Gibco, USA) containing penicillin-streptomycin (Gibco), and 10% fetal bovine serum (Gibco) in a humidified atmosphere of 95% air and 5% CO2 at 37 °C.

2.2. ^{18}F-FDG and ^{11}C-acetate uptake and cell growth

Cells were seeded on 6-well plates (1.5×10^5 cells per well) overnight. Before the uptake experiments, the medium was changed to a glucose-free medium (Gibco). Approximately 0.037 MBq of ^{18}F-FDG and 0.37 MBq of ^{11}C-acetate were added to the cells and incubated for 20 min. The cells were washed with phosphate-buffered saline (PBS), and then lysis buffer (1% SDS and 60 mM Tris-HCl in distilled water) were added to each well. The lysed cells were then harvested to measure the amount of radioactivity using a gamma-counter (Perkin Elmer, USA). The measured radioactivity was divided by the radioactivity added and normalized to protein content using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Cell growth rates were determined by seeding 1.5×10^5 cells in 6-well plates in an appropriate medium for 3 days. The cells were counted using 0.4% trypan blue stain (BIORAD, United Kingdom).

2.3. Chemicals

HBSS for nutrient starvation was purchased from GIBCO. CQ as a late-phase inhibitor of autophagy was used at a final concentration of 20 μM for 2 h. It was purchased from Sigma-Aldrich.

2.4. Western blot analysis

After chemical treatment, cells were washed with PBS and lysed with 1% SDS lysis buffer with protease inhibitor (Roche, Germany). Each sample were separated by SDS-PAGE, and transferred onto PVDF membranes (Millipore, USA). Following incubation with primary antibodies against p62 (Santa-Cruz, sc28359, USA), LC3B(2775), mTOR(2972), phospho-mTOR(2971), AMPK(2603), phospho-AMPK(2535) (Cell Signaling, USA), and actin (Sigma-Aldrich, A1978, USA), membranes were incubated with goat anti-rabbit (SC2004) or anti-mouse (SC2004) IgG-horseradish peroxidase (Santa Cruz) as the secondary antibody. Labeled specific protein bands were visualized using ECL Kit (Thermo, USA).

2.5. RNA extraction and quantitative polymerase chain reaction (qPCR)

Total RNA from cells subjected to various experimental conditions was isolated using an RNeasy mini kit (Qiagen, Germany). One microgram of total RNA was used to synthesize cDNA with a QuantiTech reverse transcription kit (Qiagen, Germany) according to the manufacturer's instructions. After then, samples were analyzed for G6Pase or PEPCK by SYBR premix Ex Taq using a Takara thermal cycler real-time system (Takara, Japan). Each cDNA was amplified (95 °C for 5 s, 58 °C for 10 s, and 72 °C for 20 s, for 40 cycles) using specific primers [G6Pase: forward primer 5′-TGTCA CGAATCTACCTTGCTGCTC-3′, reverse primer 5′-TATAGATGCTGTGG ATGTGGCTGAA-3′; PEPCK: forward primer 5′-GGCATTATCTTTG-GAGGCCGTAG-3′, reverse primer 5′-GCCAGGTATTTGCCGAAGTTG-TAG-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward primer 5′-GCACCGTCAAGGCTGAGAAC-3′, reverse primer 5′-TGGTGAAGACGCCAGTGGA-3′].

2.6. Reverse transcriptase PCR (RT-PCR) analysis

Total RNA was prepared as described above. PCR involved 25 cycles of annealing, extension, and denaturation. The annealing step consisted of 30 s at 52 °C for p62 and actin. The following primers were used (p62: forward primer 5′-CTGCCCA-GACTACGACTTGTGT-3′, reverse primer 5′-TCAACTTCAATGCCCA-GAGG-3′; and actin: forward primer 5′-ATCTGGCACCACACC TTCTACAATGAGCTGCG-3′, reverse primer 5′-ACACCAGACATAGTA GCAGAAATCAAG-3′).

2.7. Inhibition of autophagy by transfection of shRNAs

Beclin1, LC3B, and scrambled control shRNAs were purchased from Santa Cruz. Scrambled control shRNA was used as a negative control. Transfection of LC3B, Beclin 1, or scrambled control shRNAs into the cells was performed using the Neon Transfection System (Invitrogen, USA). Briefly, the cultured cells were trypsinized and suspended in PBS. Approximately 5 × 106 cells were centrifuged for 10 min and washed with PBS. The pellet was suspended in 10 μL Transfection Resuspension Buffer R and mixed with shRNA-containing cells and the shRNA mixture in the Neon Tube containing 3 mL Neon Electrolytic Buffer E in the Neon Pipette Station. Cells were pulsed once with a voltage of 1400 and a width of 20. After the pulse, cells were quickly transferred into a culture plate containing culture medium.

2.8. Statistical analysis

Each experiment was carried out in triplicate, and quantitative data were expressed as the mean ± S.D. Statistical analysis was conducted using the Sigma plot analysis program.

3. Results

3.1. Characterization of glycolysis and gluconeogensis in HCC cell lines

Since human HCC tumors showed the different uptake of glucose and acetate [5], we first characterized the metabolic patterns of 2 different HCC cell lines (HepG2 and Hep3B). As shown in Fig. 1A and B, HCC cell lines showed different uptake patterns of ^{18}F-FDG and ^{11}C-acetate. Hep3B cells displayed the most ^{18}F-FDG uptake but relatively low ^{11}C-acetate uptake, whereas HepG2 cells showed the lowest ^{18}F-FDG uptake and the highest ^{11}C-acetate uptake (Fig. 1A). As we expected, similar to human tumors, Hep3B cells with the highest glycolytic activity showed more proliferation compare with HepG2 cells with the lowest glycolytic activity (Fig. 1B). The glycolytic phenotype appears to be related to favorable growth in cancer cells.

3.2. Starvation induces autophagy and gluconeogenesis in low glycolytic HepG2 cells but not in glycolytic Hep3B cells

To determine the relation between autophagy and gluconeogenesis in glycolytic or low glycolytic HCC cell lines, first, we established starvation-induced autophagy condition with incubation in HBSS. Both HCC cell lines showed typical responses to starvation, including decreased mammalian target of rapamycin (mTOR) and phosphor-mTOR, and activated adenosine monophosphate-activated protein kinase (AMPK) by incubation in HBSS (Fig. 2A and B). In addition, there were changes in the amounts of microtubule-associated protein light chain 3A and B (LC3A/B), along with a decrease in autophagic substrate, p62, over the period of starvation, indicating the activation of autophagy in both HepG2 and Hep3B cells (Fig. 2C). The level of autophagy substrate p62 was decreased as intense as autophagic flux was increased that is typical phenomena in cell and used as method to detect level of autophagic flux. To ensure that the reduction of p62 was not due to the decreased transcription of p62 gene and was by increased autophagic flux, we evaluated the level of p62/SQSTM1 (sequestosome1) gene expression. As shown in Fig. 2D, the p62 gene was increased in HepG2 cells but unchanged in Hep3B cells. We observed that autophagy was well activated by starvation using HBSS.

Next, we checked the expression level of gluconeogenesis related gene G6pase and PEPCK that are widely known as rate-limiting enzymes in gluconeogenesis. We focused on the expression changes of G6pase and PEPCK in HepG2 and Hep3B cells under starvation-induced autophagy condition. As shown in Fig. 2F, the expression of G6Pase was significantly elevated, up to 11 folds, in HepG2 cells. In contrast, Hep3B cells showed extremely low levels of G6Pase in normal condition (Fig. 2E) as well as in starvation (Fig. 2G). Likewise, PEPCK expression levels continuously increased over the starvation period in HepG2 cells but were only mildly increased in Hep3B cells (Fig. 3G). These results were evident that autophagy was induced in HepG2 and Hep3B cells upon starvation but regulation of gluconeogenic gene expression was well preserved in low glycolytic HepG2 cells but not in glycolytic Hep3B cells.

3.3. Inhibition of autophagy decreases gluconeogenesis in low glycolytic HepG2 cells

The contribution of autophagy to gluconeogenesis was evaluated using an autophagy blocker, chloroquine (CQ). This lysosomal blocker inhibits protein degradation in the autolysosome after fusion of autophagosme and lysosome. HepG2 and Hep3B cells were cultured in HBSS to activate starvation-induced gluconeogenesis and incubated with 20 μM of CQ for 2 h before the end of each time point. Western blot analysis showed accumulation of LC3B by CQ treatment, which ensured successful inhibition of autophagy (Fig. 3A).

In HepG2 cells, CQ was able to block autophagy, which in turn reduced starvation-induced gluconeogenesis. The levels of G6Pase and PEPCK expression were significantly suppressed by CQ and these suppression effects were continuously maintained until the last time point of the experiment (Fig. 3B). However, in Hep3B cells, incubation with HBSS over 24 h did not significantly induced the expression of gluconeogenic genes and the levels of G6Pase and PEPCK expression did not differ, even after inhibition of autophagy by CQ. Neither induction of autophagy nor inhibition of autophagy seemed to have any remarkable effect on the expression of gluconeogenic genes (Fig. 3B).

To confirm the role of gluconeogenesis inhibition by autophagy blocking, we knock downed two autophagy-related genes using short hairpin RNAs (shRNAs) of Beclin 1 and LC3B. In both HepG2 and Hep3B cells, the transfection of shRNAs resulted in significant decrease in the levels of the respective protein (Fig. 4A). Autophagy related gene knock downed cells were cultured in HBSS to induce gluconeogenesis, and qRT-PCR was performed to measure the expression levels of G6Pase and PEPCK. LC3B knockdown caused a decrease in the HBSS induced expression of G6Pase and PEPCK in

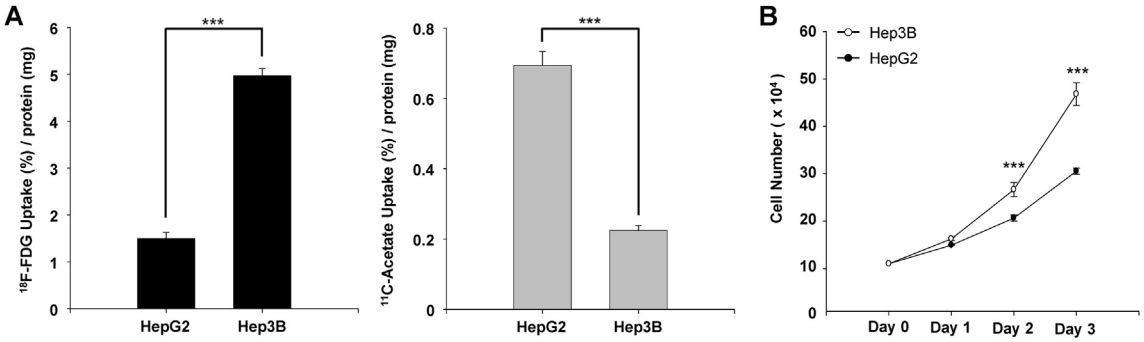

Fig. 1. Patterns of metabolic radiotracer uptake and growth rates in HCC cell lines. (A) The level of ^{18}F-FDG and ^{11}C-acetate uptake was measured using a gamma-counter. Hep3B cells displayed dominant ^{18}F-FDG but low ^{11}C-acetate uptake. In contrast, HepG2 cells showed the lowest ^{18}F-FDG but the highest ^{11}C-acetate uptake (B) Hep3B cells with higher ^{18}F-FDG-uptake showed more rapid cell growth rates. Data are represented as means ± SD. ***P < 0.001.

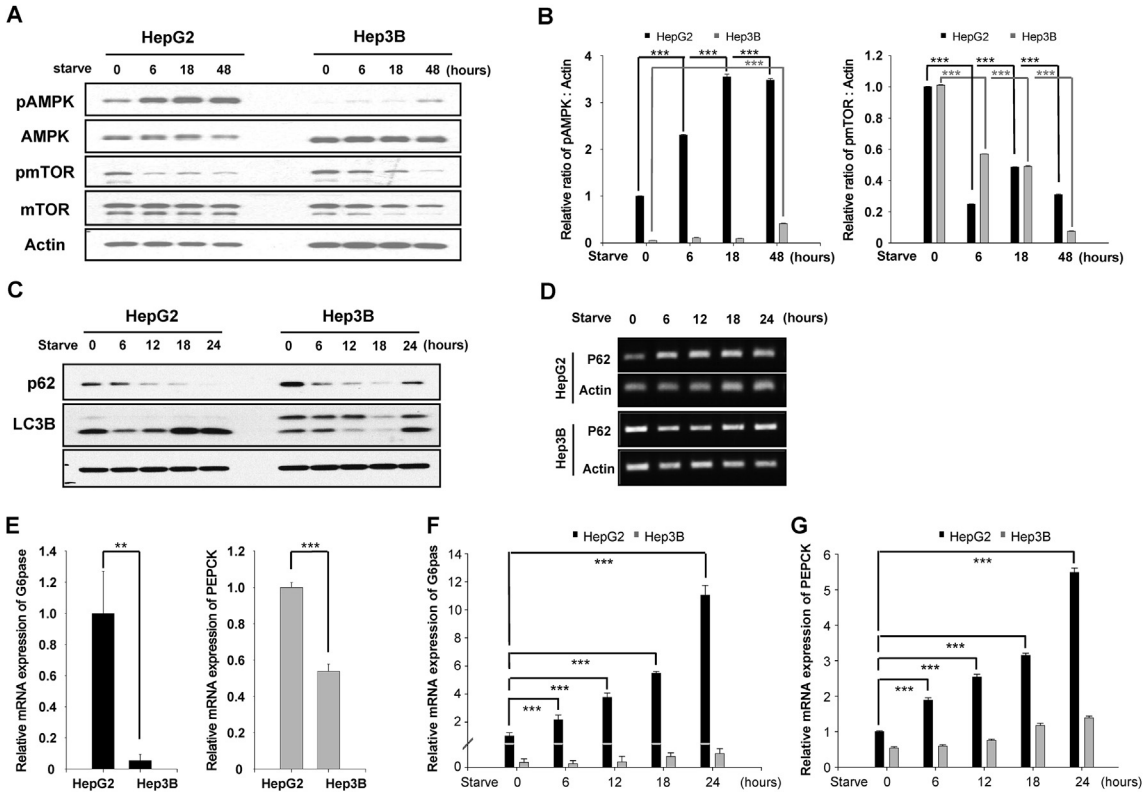

Fig. 2. Starvation induced autophagy and gluconeogenesis. HepG2 and Hep3B cells were incubated in HBSS to mimic starve culture condition for the indicated times. (A) The levels of phosphorylated or normal AMPK and mTOR representing starvation conditions were detected using western blot analysis and (B) the changing level of both protein activations were expressed as numerical values that calculated the intensity of panel A. (C) LC3B indicates the level of autophagy and p62 was used as an autophagy substrate. (D) The mRNA levels of p62 were analyzed by RT-PCR. (E) G6Pase and PEPCK mRNA levels were measured as rate-limiting enzymes of gluconeogenesis. (F) G6pase mRNA and (G) PEPCK mRNA levels were observed under HBSS treated culture condition in a time dependent manner. Data are expressed as the means ± standard deviation (SD). $^{**}P < 0.01$, $^{***}P < 0.001$.

HepG2 but not in Hep3B cells. The knockdown of Beclin 1 also reduced the HBSS induced expression of G6Pase and PEPCK compared to controls in HepG2 cells (Fig. 4B). As in the chemical inhibition of autophagy by CQ, both G6Pase and PEPCK levels in Hep3B cells were relatively unchanged after HBSS, regardless of LC3B or Beclin 1 knockdown (Fig. 4B). In conclusion, starvation with

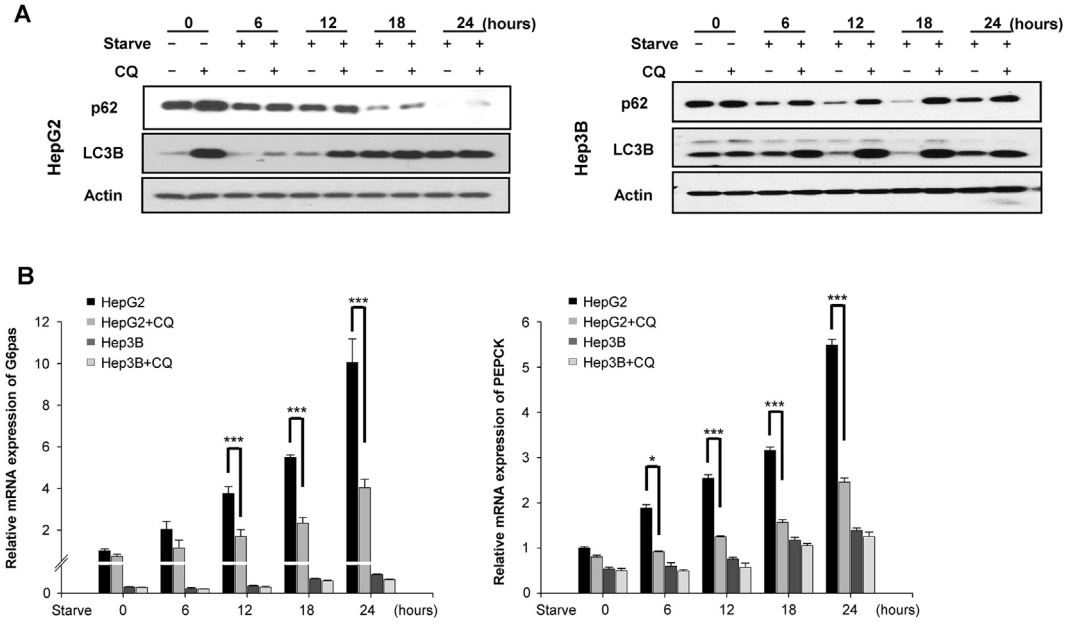

Fig. 3. Inhibition of autophagy significantly reduces expression of gluconeogenic genes. (A) HBSS was added to HepG2 and Hep3B cells for the indicated times followed by 20 μM of CQ into the same HCC culture plate 2 h before the preparation of cell lysates. Autophagy was analyzed by western blot analysis. (B) G6pase and PEPCK mRNA levels were measured by qPCR in the same condition. The increased expression levels of G6PAse and PEPCK were significantly reduced by CQ in HepG2 cells. However, these effects of CQ on the expression levels of G6PAse and PEPCK were not remarkable in Hep3B cells. Data are expressed as the means ± standard deviation (SD). $^{*}P < 0.05$, $^{***}P < 0.001$.

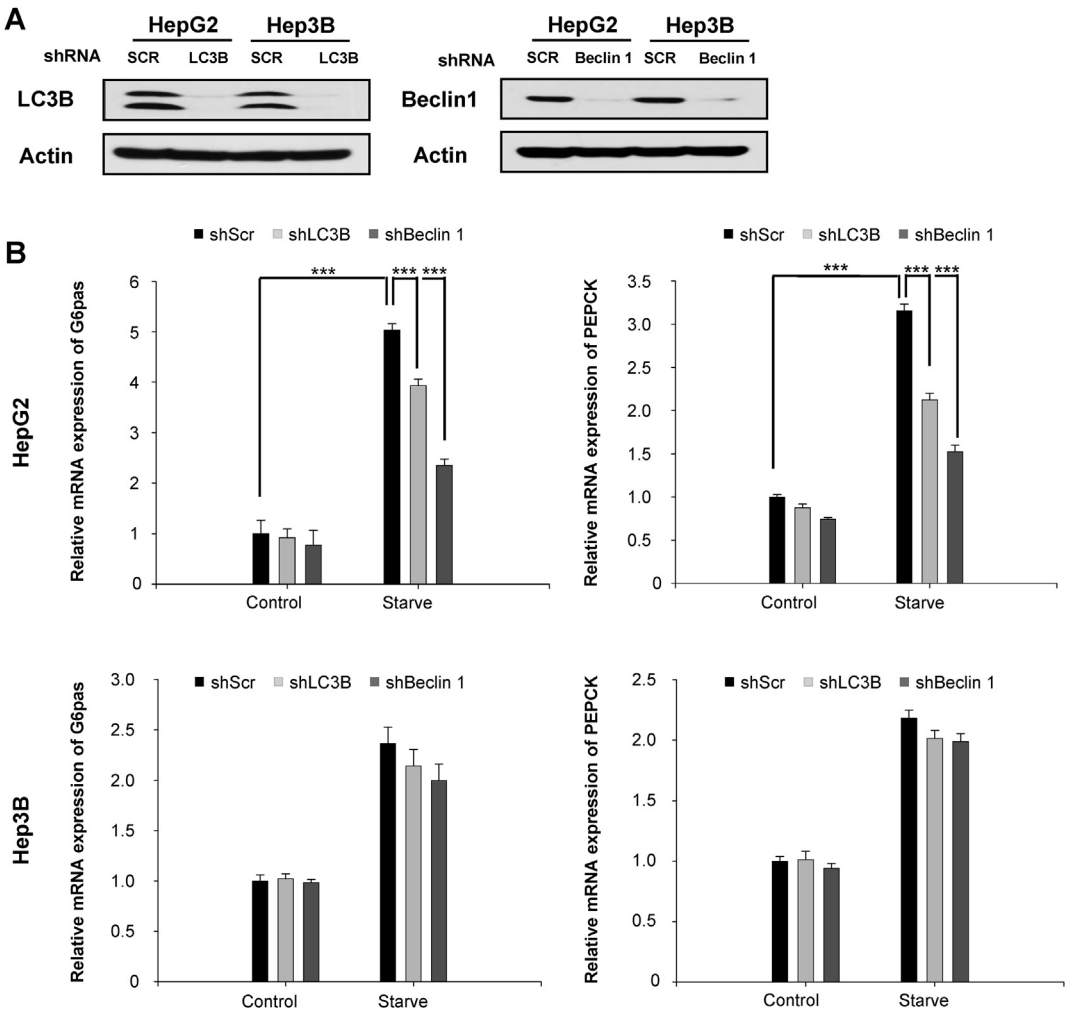

Fig. 4. Genetic inhibition of autophagy significantly affects gluconeogenic gene expression. (A) HepG2 and Hep3B cells were transfected with scrambled shRNA vector or respective shRNA vectors (LC3B or Beclin 1). Western blot analysis showed decreased expression of each protein. (B) shRNA-transfected HCC cell lines were incubated with HBSS for 18 h followed by measurements of G6pase and PEPCK mRNA levels by qPCR. The increased G6Pase and PEPCK mRNA levels were significantly reduced in HepG2 cells. However, these effects of LC3B or Beclin1 knockdown on the expression levels of G6Pase and PEPCK were not remarkable in Hep3B cells. Data are expressed as the means ± standard deviation (SD). ***$P < 0.001$.

HBSS induced autophagy in both low glycolytic HepG2 and glycolytic cancer Hep3B cells but only low glycolytic HepG2 cells showed increasing the levels of gluconeogenic gene expression that suppressed by autophagy inhibition.

4. Discussion

Aerobic glycolysis in cancer cells regardless of oxygen availability was first described by Otto Warburg; this so-called Warburg effect is considered one of the hallmarks of cancer [14]. With the advent of modern imaging techniques such as PET, radiolabeling of glucose with ^{18}F has successfully imaged the altered metabolism of cancer, revolutionizing conventional cancer diagnosis [15]. What is even more important is that the degree of glycolysis on PET is associated with biological aggressiveness and patient survival outcome [16]. The more glycolytic the tumor is, the worse the prognosis is.

In contrast to the Warburg effect, some malignant tumors show without glycolysis [17,18]. Other than glucose analogues, alternative nutrient sources such as amino acids or acetate have been used for their cell metabolism [19]. HCC is one of the tumors in which alternative patterns of metabolism between glucose and acetate are vigorously studied.[5] Uptake pattern of glucose is more dominant in poorly differentiated HCCs than acetate for the detection in well differentiated HCCs [5].

HepG2 cells were one of well differentiated cancer cell line mentioned upon which is uptake acetated prefer than glucose and the growth rate was lower than glycolytic Hep3B cells (Fig. 1). Our study begun to understand why these cells use acetates instate of glucoses as nutrient source to maintain cell growth, survival and intracellular metabolism Although most cancer cells mainly use glucose as nutrient source as it is comfortable to make biomaterials, low glycolytic HepG2 cells use acetate mainly.

We thought the reason of these phenomena as HepG2 may conserve gluconeogenic characteristic which is represented function of liver. HepG2 cells were originated liver cancer that has possibility of maintenance of gluconeogenic function. If that, HepG2 cells tried to produce glucose rather than uptake glucose under starved condition.

So we confirmed the expression level of gluconeogeic genes, G6pase and PEPCK because both genes are well known rate limiting enzyme in gluconeogenesis. HepG2 and Hep3B cells were incubated with HBSS to mimic starved condition and assessed the change of gluconeogenesis. In HepG2 cells, both G6Pase and PEPCK

showed remarkable increases in mRNA expression upon HBSS incubation compared to controls, whereas they were low in controls as well as in HBSS for Hep3B cells (Fig. 2). Gluconeogenesis was well preserved in low glycolytic HepG2 cells but not in glycolytic Hep3B cells. When the concept of altered carbohydrate metabolism during hepatic carcinogenesis to glycolysis is taken into consideration, HepG2 cells appeared to be metabolically similar to the normal liver by preserving gluconeogenesis upon starvation. G6Pase activity, reflecting gluconeogenesis, was well preserved in normal mouse liver but was almost completely absent in HCCs [20]. Torizuka et al. also reported high G6Pase activity in low-grade HCCs without increased FDG uptake [8].

Next, how HepG2 get biomaterials for cell growth and survival if HepG2 cells cannot use glucose for the innate role of gluconeogenesis. As shown in Fig. 1A, HepG2 cells used acetate as a nutrient source. Especially under starved culture condition with HBSS, HepG2 could not be supported with enough nutrient source that produce biomaterials for cell growth and survival. In this condition, autophagic flux would be increased to support biomaterials because autophagy has recycling role of cellular components; damaged or unnecessary intracellular organelles those are breakdown to basic biomasses using lysosomal degradation. We confirmed increasing autophagic flux in a time dependent manner by HBSS starvation (Fig. 2C). In addition, to further confirm the contribution of autophagy in gluconeogenesis, we inhibited autophagy using CQ and knockdown of two autophagy-related genes using shRNAs of Beclin1 and LC3B. CQ was able to block autophagy, which in turn reduced gluconeogenesis in HepG2 cells (Fig. 3B). The results were consistent with the knockdown of Beclin 1 and LC3B causing a reduction in HBSS-induced expression of G6Pase and PEPCK in HepG2 cells (Fig. 4B). However, In Hep3B cells, the low levels of G6Pase and PEPCK expression did not differ, regardless of the induction of autophagy or blocking of autophagy with the chemical or genetic modification (Figs. 3G and 4B).

HBSS-induced autophagy was observed in both HepG2 and Hep3B cells, but only gluconeogenesis was seen in HepG2 cells. The role of autophagy in cancer can differ by the genetic composition or altered tumor microenvironment [21]. Autophagy plays a role in preserving energy and nutrients to ensure long-term survival when there is limited external nutrient supply [22]. Upon the activation of autophagy, cancer cells suppress proliferation and motility to induce dormancy with recovery capabilities [23]. In this study, HepG2 and Hep3B cells showed typical responses to starvation, including decreased phospho-mTOR and activated phospho-AMPK levels, resulting in autophagy induction. However, the same HBSS-induced autophagy was accompanied by gluconeogenesis only in HepG2 cells but not in Hep3B cells. Autophagy supplied metabolites for gluconeogenesis in cancer cells seemed incompatible with its well-known energy and nutrient saving function. In contrast to the glycolytic phenotype of cancer cells, such as Hep3B cells, HepG2 cells had low glycolytic activity but preserved gluconeogenesis upon fasting by aid of autophagy. The liver is known to recycle amino acid substrates from autophagic proteolysis for gluconeogenesis to maintain blood glucose levels in fasting animals [12]. Therefore, the findings suggest that gluconeogenesis may contribute to low glycolytic features in HepG2 cells and liver and autophagy acts important role control gluconeogenesis.

In this study, we confirmed the alternative uptake pattern of glucose for glycolysis and acetate in human hepatocellular carcinoma cell lines. In contrast to typical glycolytic tumors, low glycolytic tumors were still able to preserve hepatic gluconeogenesis with autophagy as a supporting mechanism. The metabolic similarity of low glycolytic tumors to the liver is consistent with known, less aggressive clinical characteristics of these tumors.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning (2012R1A1A3008042), and by a faculty research grant from Yonsei University College of Medicine for 2013 (6-2013-0050).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.103.

References

[1] M. Thomas, Molecular targeted therapy for hepatocellular carcinoma, J. Gastroenterol. 44 (Suppl. 19) (2009) 136–141.
[2] L. Rimassa, A. Santoro, The present and the future landscape of treatment of advanced hepatocellular carcinoma, Dig. Liver Dis. 42 (Suppl. 3) (2010) S273–S280.
[3] A.L. Vāvere, S.J. Kridel, F.B. Wheeler, J.S. Lewis, 1-11C-acetate as a PET radiopharmaceutical for imaging fatty acid synthase expression in prostate cancer, J. Nucl. Med. 49 (2008) 327–334.
[4] M. Yun, S.H. Bang, J.W. Kim, J.Y. Park, K.S. Kim, J.D. Lee, The importance of acetyl coenzyme A synthetase for 11C-acetate uptake and cell survival in hepatocellular carcinoma, J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 50 (2009) 1222–1228.
[5] J.-W. Park, J.H. Kim, S.K. Kim, K.W. Kang, K.W. Park, J.-I. Choi, W.J. Lee, C.-M. Kim, B.H. Nam, A prospective evaluation of 18F-FDG and 11C-acetate PET/CT for detection of primary and metastatic hepatocellular carcinoma, J. Nucl. Med. 49 (2008) 1912–1921.
[6] C.L. Ho, S. Chen, D.W. Yeung, T.K. Cheng, Dual-tracer PET/CT imaging in evaluation of metastatic hepatocellular carcinoma, J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 48 (2007) 902–909.
[7] M. Nakajo, Y. Kajiya, A. Tani, S. Yoneda, H. Shirahama, M. Higashi, M. Nakajo, (1)(8)FDG PET for grading malignancy in thymic epithelial tumors: significant differences in (1)(8)FDG uptake and expression of glucose transporter-1 and hexokinase II between low and high-risk tumors: preliminary study, Eur. J. Radiol. 81 (2012) 146–151.
[8] T. Torizuka, N. Tamaki, T. Inokuma, Y. Magata, S. Sasayama, Y. Yonekura, A. Tanaka, Y. Yamaoka, K. Yamamoto, J. Konishi, In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET, J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 36 (1995) 1811–1817.
[9] F.Q. Nuttall, A. Ngo, M.C. Gannon, Regulation of hepatic glucose production and the role of gluconeogenesis in humans: is the rate of gluconeogenesis constant? Diabetes Metab. Res. Rev. 24 (2008) 438–458.
[10] T. Ueno, J. Ezaki, E. Kominami, Metabolic contribution of hepatic autophagic proteolysis: old wine in new bottles, Biochim. Biophys. Acta 1824 (2012) 51–58.
[11] Y. Li, J. Zhang, X. Chen, T. Liu, W. He, Y. Chen, X. Zeng, Molecular machinery of autophagy and its implication in cancer, Am. J. Med. Sci. 343 (2012) 155–161.
[12] J. Ezaki, N. Matsumoto, M. Takeda-Ezaki, M. Komatsu, K. Takahashi, Y. Hiraoka, H. Taka, T. Fujimura, K. Takehana, M. Yoshida, J. Iwata, I. Tanida, N. Furuya, D.M. Zheng, N. Tada, K. Tanaka, E. Kominami, T. Ueno, Liver autophagy contributes to the maintenance of blood glucose and amino acid levels, Autophagy 7 (2011) 727–736.
[13] J. Onodera, Y. Ohsumi, Autophagy is required for maintenance of amino acid levels and protein synthesis under nitrogen starvation, J. Biol. Chem. 280 (2005) 31582–31586.
[14] P.P. Hsu, D.M. Sabatini, Cancer cell metabolism: Warburg and beyond, Cell 134 (2008) 703–707.
[15] G.Z. Xu, C.Y. Li, L. Zhao, Z.Y. He, Comparison of FDG whole-body PET/CT and gadolinium-enhanced whole-body MRI for distant malignancies in patients with malignant tumors: a meta-analysis, Ann. Oncol. Off. J. Eur. Soc. Med. Oncology/ESMO 24 (2013) 96–101.
[16] F.F. Teng, X. Meng, X.D. Sun, J.M. Yu, New strategy for monitoring targeted therapy: molecular imaging, Int. J. Nanomed. 8 (2013) 3703–3713.
[17] C.-L. Ho, C. Simon, D.W. Yeung, 11C-acetate PET imaging in hepatocellular carcinoma and other liver masses, J. Nucl. Med. 44 (2003) 213–221.
[18] D.E. Kang, R.L. White Jr., J.H. Zuger, H.C. Sasser, C.M. Teigland, Clinical use of fluorodeoxyglucose F 18 positron emission tomography for detection of renal cell carcinoma, J. Urol. 171 (2004) 1806–1809.
[19] J. McConathy, M.M. Goodman, Non-natural amino acids for tumor imaging using positron emission tomography and single photon emission computed tomography, Cancer Metastasis Rev. 27 (2008) 555–573.

[20] B. Wang, S.H. Hsu, W. Frankel, K. Ghoshal, S.T. Jacob, Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha, Hepatology 56 (2012) 186–197.

[21] E.J. White, V. Martin, J.L. Liu, S.R. Klein, S. Piya, C. Gomez-Manzano, J. Fueyo, H. Jiang, Autophagy regulation in cancer development and therapy, Am. J. Cancer Res. 1 (2011) 362–372.

[22] K. Sato, K. Tsuchihara, S. Fujii, M. Sugiyama, T. Goya, Y. Atomi, T. Ueno, A. Ochiai, H. Esumi, Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation, Cancer Res. 67 (2007) 9677–9684.

[23] E. White, R.S. DiPaola, The double-edged sword of autophagy modulation in cancer, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 15 (2009) 5308–5316.