Oncology Research, Vol. 25, pp. 939–946
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504016X14809827856524
E-ISSN 1555-3906
www.cognizantcommunication.com

Histone Acetyltransferase 1 Promotes Cell Proliferation and Induces Cisplatin Resistance in Hepatocellular Carcinoma

Xin Jin,* Shenghua Tian,† and Pingping Li‡

*Department of Digestive Surgical Oncology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China
†Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China
‡Department of Endocrine and Vascular Surgery, Taihe Hospital, Hubei Medical College, Shiyan, Hubei, P.R. China

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the world. Mutations, overexpression, and improper recruitment of HATs can lead to tumorigenesis. HAT1 is the first histone acetyltransferase identified and is related with developing HCC, but the mechanism is still unclear. Interestingly, we found that HAT1 was upregulated in HCC patient specimens and showed that its upregulation facilitates HCC cell growth in vitro and in vivo. Moreover, we demonstrated that HAT1 promoted glycolysis in HCC cells and knockdown of HAT1 sensitized HCC cells to apoptotic death induced by cisplatin. Our results suggest that HAT1 might act as an oncogenic protein promoting cell proliferation and inducing cisplatin resistance in HCC, and targeting HAT1 represents a viable strategy for effective treatment of advanced HCC.

Key words: Hepatocellular carcinoma (HCC); Histone acetyltransferase 1 (HAT1); Cell proliferation; Warburg effect; Cisplatin; Drug resistance

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the world[1] and is considered to be a global health threat[2]. Its incidence rate has increased worldwide during the past two decades, especially in Asian and African countries due to a higher rate of HBV and HCV infections[3–5]. Hepatic resection and liver transplantation result in the best clinical outcome in patients with well-defined tumors; however, the 5-year survival rate remains at only 60%–70%[1]. Patients with HCC are usually asymptomatic and diagnosed at late stages when surgical treatment is no longer suitable. Unfortunately, HCC has been considered to be a relatively chemotherapy refractory tumor. It can be difficult to achieve benefits from chemotherapy in patients with advanced HCC[6]. Therefore, there is an urgent need to develop novel therapeutics to treat HCC.

Histone-related proteins [histone acetyltransferases and histone deacetylases (HDACs)] can influence DNA transcription through the balance between histone acetylation and deacetylation[7]. It has been known that mutations, overexpression, and improper recruitment of HATs lead to developing malignant tumors[8]. Histone acetyltransferase 1 (HAT1) is the first histone acetyltransferase identified and is one of the most poorly understood members of this family[9]. Some evidence indicates that HAT1 is related to developing HCC, but the mechanism is still unclear[10].

To date, few studies have examined the role of HAT1 in HCC. In the present study, we studied HAT1 expression in HCC patient samples and demonstrated that HAT1 was upregulated in HCC patient specimens. Furthermore, we showed that HAT1 regulated HCC cell growth in vitro and in vivo. Moreover, HAT1 regulates glucose metabolism in HCC cells, and knockdown of HAT1 sensitizes HCC cells to apoptotic death induced by cisplatin. Collectively, these results are contributing to a better understanding of the role of HAT1 in HCC and may also shed new light on developing novel therapeutic strategies for HCC.

MATERIALS AND METHODS

Cell Lines and Cell Culture

The HCC cell lines HepG2 and SK-Hep-1 were purchased from the Chinese Academy of Science Cell Bank and cultured in a 5% CO_2, 37°C, and 95% humidity

Address correspondence to Xin Jin, Department of Digestive Surgical Oncology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1227 Jiefang Road, Wuhan, Hubei 430022, P.R. China. E-mail: jinxinunion@gmail.com

incubator. HepG2 and SK-Hep-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and 100 U/ml penicillin and 100 μg/ml streptomycin (Thermo Fisher Scientific).

Reagents

Anti-HAT1 (1:1,000 dilution) was purchased from Abcam (Cambridge, MA, USA), anti-PARP (1:1,000 dilution) and anti-cleaved PARP (1:1,000 dilution) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA), and anti-β-tubulin (1:5,000 dilution) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cisplatin was from Sigma-Aldrich (St. Louis, MO, USA).

Western Blot

Cells were lysed with lysis buffer (1% Nonidet P-40, 1× PBS, 0.1% sodium dodecyl sulfate, and 1% protease inhibitor cocktail) followed by protein quantification by the BCA method. Samples were diluted in loading buffer containing DTT and boiled for 5 min. Equal amounts of protein for each sample were separated by 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were immunoblotted with specific primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies and visualized by SuperSignal West Pico Stable Peroxide Solution (Thermo Fisher Scientific).

Real-Time RT-PCR

Total RNA was extracted from cells using TRIzol reagent (Thermo Fisher Scientific). The cDNA was synthesized using SuperScript III reverse transcriptase (Thermo Fisher Scientific). Real-time PCR was performed using IQ SYRB Green Supermix and an iCycleriQTX detection system (Bio-Rad, Hercules, CA, USA). All signals were normalized against GAPDH, and the $2^{-\Delta Ct}$ method was used to quantify the fold change. Primers used were as follows: *FBP1*, 5′-ACAGCAGTCAAAG CCATCTC-3′ (forward) and 5′-GGTTCCACTATGATG GCGTG-3′ (reverse); *PKM2*, 5′-TCGCATGCAGCAC CTGATT-3′ (forward) and 5′-CCTCGAATAGCTGC AAGTGGTA-3′ (reverse); *GLUT1*, 5′-CATCATCTTC ATCCCGGC-3′ (forward) and 5′-CTCCTCGTTGCGG TTGAT-3′ (reverse); *LDHA*, 5′-GATCTCGCGCACGC TACT-3′ (forward) and 5′-CACAATCAGCTGGTCCT TGAG-3′ (reverse); *GAPDH*, 5′-ACCCACTCCTCCA CCTTTGAC-3′ (forward) and 5′-TGTTGCTGTAGCC AAATTCGTT-3′ (reverse).

RNA Interference

Lentivirus-based control and gene-specific shRNAs were purchased from Sigma-Aldrich. Transfections were performed according to the manufacturer's protocol. Cells were collected 48 h posttransfection. shRNA sequences were as follows: shHAT1#1, CCGGCGGCGTGTTATT GAACGACTTCTCGAGAAGTCGTTCAATAACA CGCCGTTTTTG; and shHAT1#2, CCGGGCTACATG ACAGTCTATAATTCTCGAGAATTATAGACTGTC ATGTAGCTTTTTG.

Caspase 3 Activity Measurement

The activity of caspase 3 was measured by Caspase 3 Colorimetric Protease Assay (Life Technologies, Carlsbad, CA, USA). Cells were counted and pelleted ($3–5\times10^6$ cells per sample). Cells were lysed with 50 μl of lysis buffer followed by protein quantification by the BCA method. Each cytosol extract was diluted to a concentration of 50–200 μg of protein per 50 μl of cell lysis buffer (1–4 mg/ml). Then 50 μl of 2× reaction buffer (containing 10 mM DTT) was added to each sample; 5 μl of the 4 mM DEVD-pNA substrate (200 μM final concentration) was added and incubated at 37°C for 2 h in the dark. Reactions were measured in a microplate reader at 405-nm wavelength.

Cell Proliferation Assay

Cell growth was monitored by absorbance using the MTS assay according to the manufacturer's instructions (Promega, Madison, WI, USA). Briefly, cells were plated in 96-well plates at a density of 1,000 cells per well. At the indicated times, 20 μl of CellTiter 96R AQueous One Solution Reagent (Promega) was added to cells. After incubating for 60 min at 37°C in the cell incubator, cell growth was measured in a microplate reader at 490 nm.

Generation and Imaging of HCC Xenografts in Mice

The 6-week-old BALB/c-nu mice were randomly divided into two groups ($n=3$ per group). All mice were housed in standard conditions with a 12-h light/dark cycle and access to food and water ad libitum. All the mice were cared for in compliance with the National Research Council's Guide for Animal Care. SK-Hep-1 cells (5×10^6) infected with lentivirus-expressing empty vector (shControl) or shHAT1 [in 100 μl of 1× PBS plus 100 μl of Matrigel (BD Biosciences, San Jose, CA, USA)] were injected subcutaneously into the right flank of mice. The tumor size was measured every other day for 21 days and calculated using the formula $L\times W^2\times0.5$. Mice were sacrificed with CO_2 asphyxiation on day 22 after injection, and tumor grafts were harvested. Tumor tissue was fixed with formalin and embedded in paraffin.

Measurements of Glucose Consumption and Lactate Production

At 24 h after plasmid transfection or 48 h after lentivirus infection, the spent medium was collected for measurement of glucose and lactate concentrations. Glucose

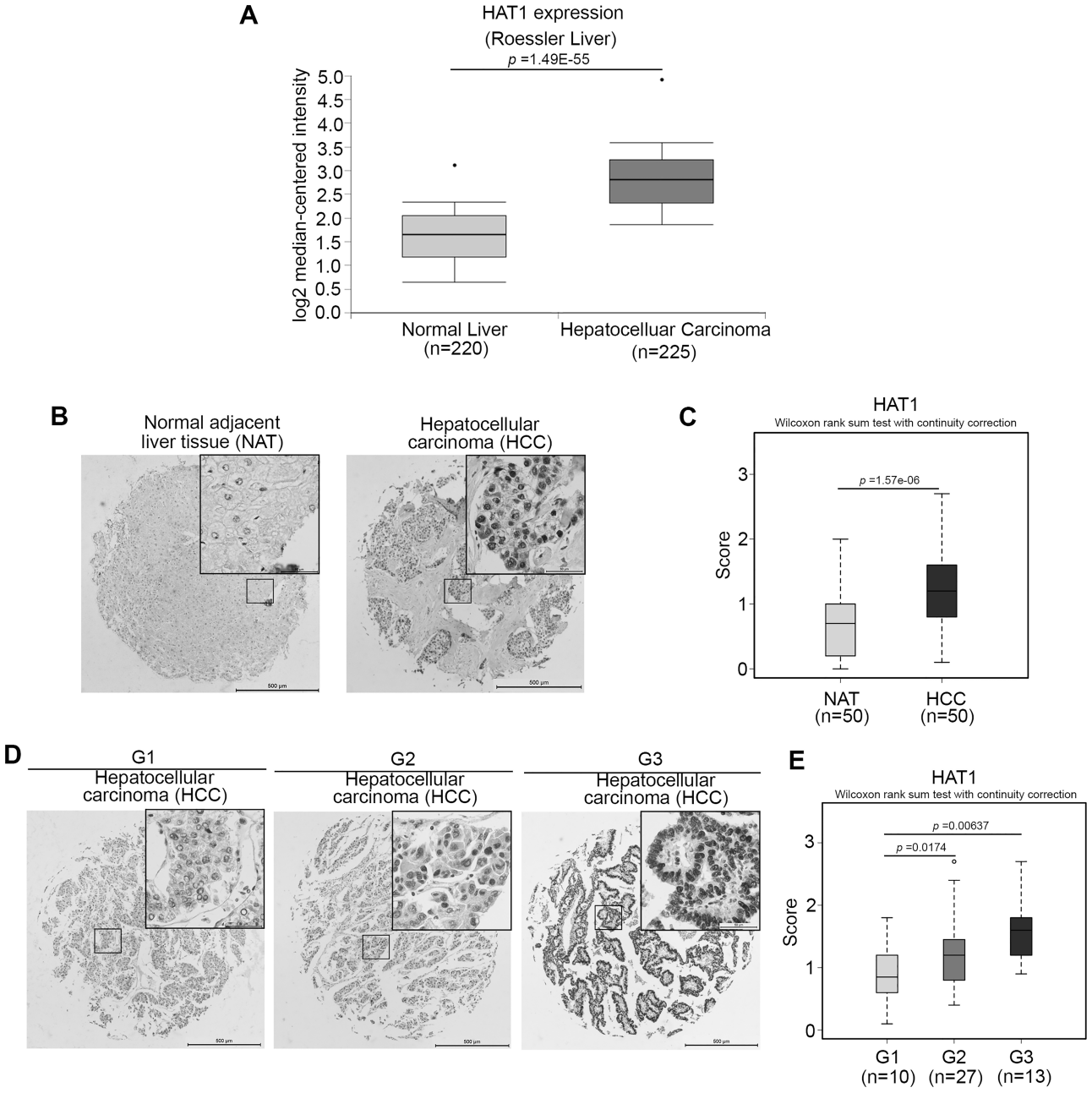

Figure 1. HAT1 is upregulated in HCC patient specimens. (A) Increased expression of HAT1 in HCC tissues ($n=220$) compared with the normal liver tissues ($n=225$) as revealed by Oncomining of the data reported by Roessler et al.[11]. (B) Representative images of IHC analysis of HAT1 protein expression on TMA ($n=50$) tissue sections. Scale bars: 500 µm. (C) Box plots of HAT1 protein expression based on their staining index (SI) in nonmalignant adjacent tissues (NAT) and HCC specimens. The p values are also shown in the graphs. (D) Representative images of IHC analysis of HAT1 protein expression on TMA ($n=50$) tissue sections. Scale bars: 500 µm. (E) Box plots of HAT1 protein expression based on their SI in HCC specimens at different clinical stages. G1, G2, and G3 represent well-differentiated, moderately differentiated, and poorly differentiated tumors, respectively. The p values are also shown.

levels were determined using a glucose (GO) assay kit (Sigma-Aldrich). Glucose consumption was the difference in glucose concentration in the spent medium when compared with unused cell culture medium. Lactate levels were determined using a lactate assay kit (Eton Bioscience, San Diego, CA, USA).

Tissue Microarray and Immunohistochemistry

The tissue microarray (TMA) slides were purchased from the Department of Pathology of Xijing Hospital, Xi'an, P.R. China (Lot No. 1202A). Based on the percentage of positive cells, the staining was scored as follows: score 0, no positive cells; score 1, 1%–25% of positive cells; score 2, 26%–50% of positive cells; score 3, >50% of positive cells. The criteria for the staining intensity were as follows: 0 for negative, 1 for low staining, 2 for media staining, and 3 for strong staining. The final staining index (SI) for each staining was obtained by multiplying values obtained from staining percentage and intensity.

Statistical Analysis

Experiments were carried out with three or more replicates unless otherwise stated. All values were expressed as means ± SD. Comparisons between two mean values were made by independent sample *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

HAT1 Is Upregulated in HCC Patient Specimens

To examine HAT1 expression in human HCC, we first analyzed their mRNA level in HCC and normal pancreas tissues using Oncomine microarray gene expression datasets. We found that the HAT1 mRNA level in liver tumors was higher than that in normal liver tissues (Fig. 1A)[11]. Next, we sought to determine the HAT1 protein levels in human HCC specimens using the TMA approach. We examined the expression of the HAT1 protein by immunohistochemistry (IHC) in HCC specimens obtained from a cohort of patients ($n = 50$ normal tumor paired TMA specimens). IHC staining was evaluated by measuring both percentage of staining-positive cells and staining intensity. The analysis showed that liver tumor tissues had a higher HAT1 expression level ($p = 1.57e{-}6$) when compared with adjacent normal tissues (Fig. 1B and C). Furthermore, the HAT1 protein level was increased with tumor stage (Fig. 1D and E). Together, these data indicate that HAT1 is upregulated in HCC patient specimens.

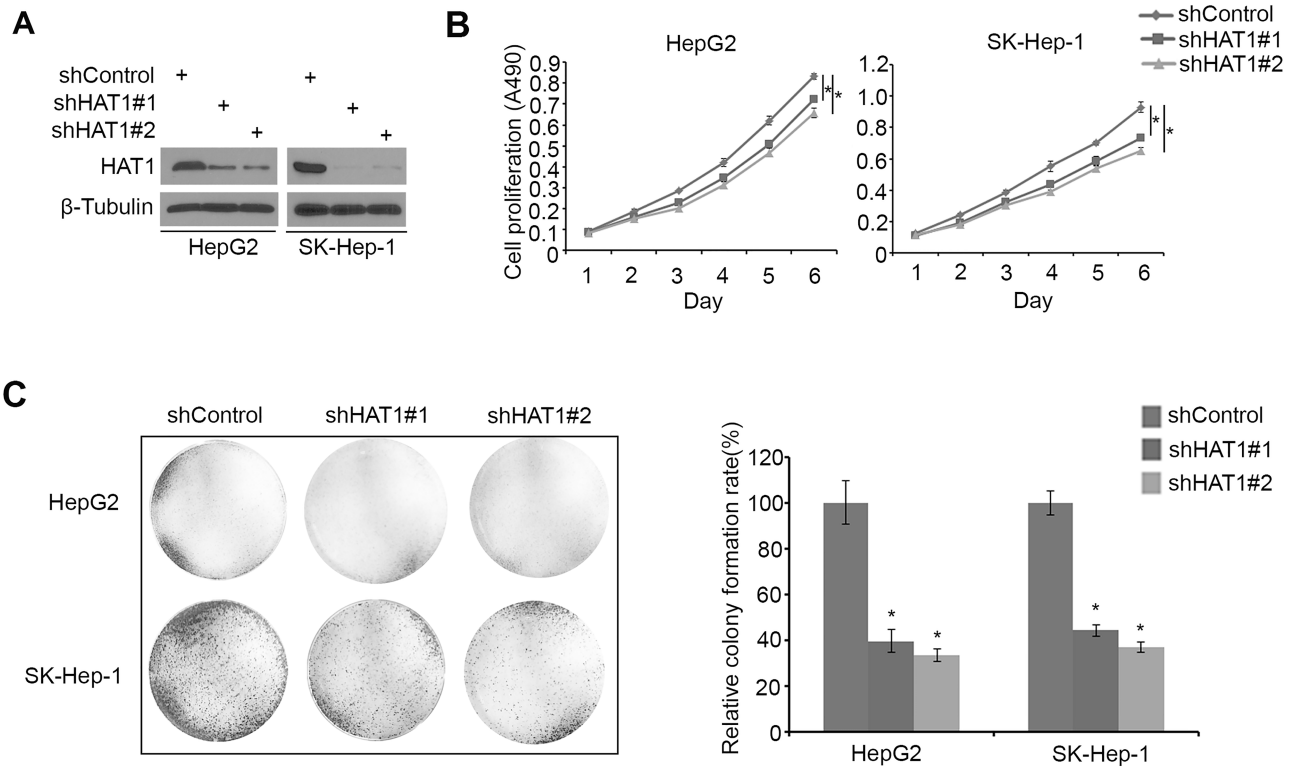

Figure 2. HAT1 promotes HCC growth in culture. HepG2 and SK-Hep-1 cells were infected with control or two independent HAT1-specific shRNAs. Forty-eight hours after transfection, cells were harvested for Western blot analysis (A), MTS assay (B), and colony formation assay (C). *$p < 0.05$.

HAT1 Promotes HCC Growth in Culture

To determine the role of HAT1 in HCC progression, we examined the effect of HAT1 protein on HCC cell growth. HepG2 and SK-Hep-1 cells were infected with control, HAT1-specific shRNAs. Western blot showed that knockdown of HAT1 was effective in both HCC cells (Fig. 2A). Cell viability and colony formation assays indicated that knockdown of HAT1 decreased cell growth in HCC cells (Fig. 2B and C). Taken together, these data suggest that HAT1 regulates HCC cell growth in vitro.

HAT1 Regulates Glucose Metabolism in HCC Cells

Increased aerobic glycolysis is a common characteristic in many human cancers, especially in HCC. Proliferative activity in HCC is closely correlated with glucose metabolism[12]. We have shown that HAT1 played a significant role in the proliferative activity of HCC. To further verify the role of HAT1 in glucose metabolism in HCC, we first examined the expression change of genes associated with glucose metabolism in HepG2 and SK-Hep-1 cells after being infected with control, HAT1-specific shRNA. We found that the mRNA level of genes associated with glycolysis, such as *LDHA*, *GLUT1*, and *PKM2*, was upregulated after knockdown of HAT1 (Fig. 3A and B). In contrast, the mRNA level of genes associated with gluconeogenesis, including *FBP1*, decreased after knockdown of HAT1 (Fig. 3A and B). Furthermore, knockdown of HAT1 decreased glucose consumption and lactate production in both HepG2 and SK-Hep-1 cells, which was consistent with gene changes in both cells (Fig. 3C and D). These results indicate that HAT1-regulated reprogramming of cancer cell metabolism might be involved, at least in part, in changing gene expression correlated with glucose metabolism in HCC cells.

Knockdown of HAT1 Sensitizes HCC Cells to Apoptotic Death Induced by Cisplatin

HCC is known to be a chemotherapy-resistant tumor[6]. Cisplatin is one of the therapeutic agents for HCC. Because of acquired or intrinsic drug resistance, cisplatin is not efficient in conferring a survival benefit in HCC patients[13]. The Warburg effect is considered to

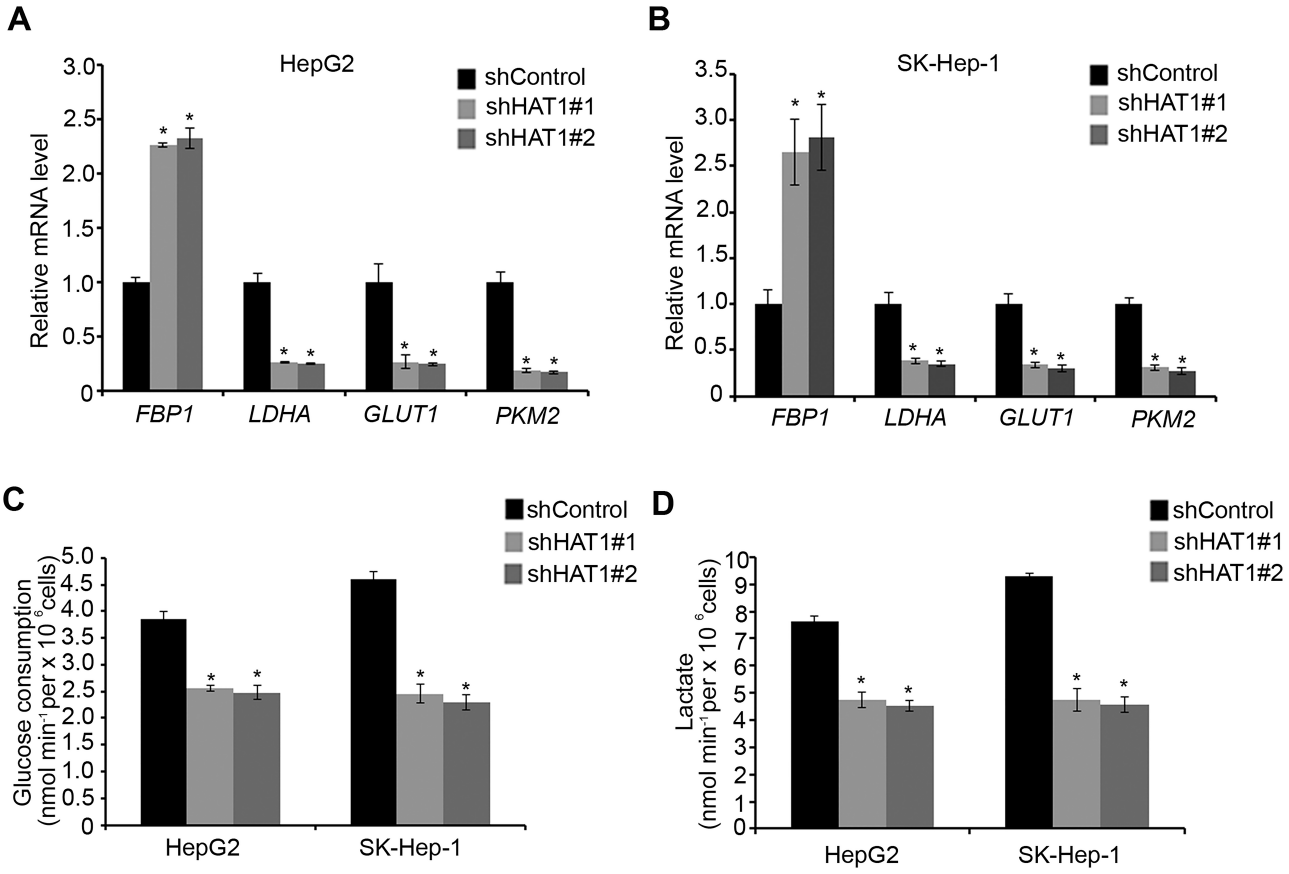

Figure 3. HAT1 regulates glucose metabolism in HCC cells. (A, B) HepG2 and SK-Hep-1 cells were infected with control or two independent HAT1-specific shRNAs. Forty-eight hours after transfection, cells were harvested for RT-qPCR analysis. *$p<0.05$. (C, D) Measurement of glucose consumption and L-lactate production in spent medium of HepG2 and SK-Hep-1 cells 48 h after transfection with indicated constructs. *$p<0.05$.

be a possible mechanism for cancer chemoresistance. Because of HAT1 being highly correlated with aerobic glycolysis in HCC, we sought to examine the role of HAT1 in cisplatin resistance. HCC cells were treated with cisplatin alone or in combination with HAT1 knockdown. We found that HAT1 knockdown increased the cleaved PARP protein level and decreased caspase 3 activity induced by cisplatin (Fig. 4A and B). MTS and colony formation assays showed that knockdown of HAT1 reduced HCC cell growth more when compared with the control group under cisplatin treatment only (Fig. 4C and D). Together, these results demonstrate that

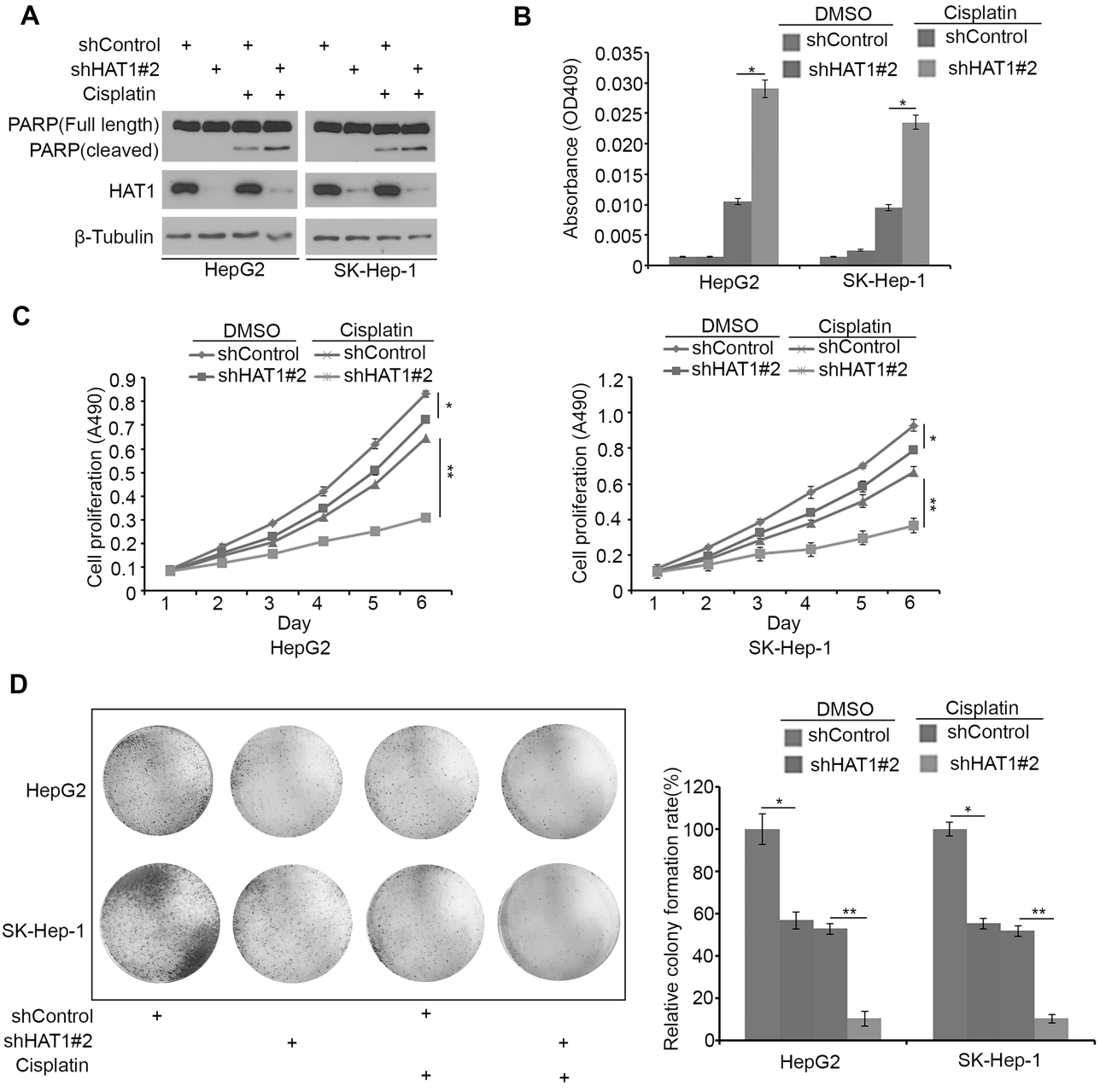

Figure 4. Knockdown of HAT1 sensitizes HCC cells to apoptotic death induced by cisplatin. HepG2 and SK-Hep-1 cells were infected with control or HAT1-specific shRNAs. Twenty-four hours after transfection, cells were treated with cisplatin (HepG2, 5 μM; SK-Hep-1, 40 μM). Forty-eight hours after transfection, cells were harvested for Western blot analysis (A). (B) Measurement of caspase 3 activity; data are means ± SD from two replicates. *$p < 0.05$. (C) Cell viability was measured by MTS assays; data are means ± SD from three replicates. *$p < 0.05$, **$p < 0.01$. (D) Equal numbers of cells and control cells were seeded onto a 60-mm dish. After 7 days, the cells were fixed and stained with crystal violet; data are means ± SD from two replicates. *$p < 0.05$, **$p < 0.01$.

knockdown of HAT1 sensitizes HCC cells to apoptotic death induced by cisplatin.

Knockdown of HAT1 Inhibits HCC Growth in Mice

To explore the role of HAT1 in HCC growth in vivo, SK-Hep-1 cells infected with control or HAT1-specific shRNAs were injected subcutaneously into the right flank of nude mice for the xenograft assay. Similarly, knockdown of HAT1 inhibited the growth of SK-Hep-1 xenografts in mice (Fig. 5A and B). The xenografts were subjected to IHC analysis for Ki-67 expression (Fig. 5C). Knockdown of HAT1 resulted in a decrease in Ki-67 staining compared with the control group (Fig. 5C and D). These results suggest that knockdown of HAT1 inhibits HCC growth in vivo.

DISCUSSION

HAT1 is a classic type B histone acetyltransferase, and it can only acetylate newly synthesized histone H4 but not nucleosomal histone[14]. It acts as an oncoprotein and is highly expressed in many cancer cells[14]. HAT1 mRNA and protein levels are elevated in primary and metastatic human colon cancer tissues[15]. Moreover, HAT1 plays an important role in the development of malignant lymphomas, and it might be an indicator for a poor prognosis in cases of diffuse large B-cell lymphoma[7]. Our data demonstrated that HAT1 was upregulated in HCC patient specimens, and HAT1 protein level was increased with tumor stage, and upregulation of HAT1 promoted HCC growth in culture and in mice. Our results suggest that HAT1 may contribute to tumorigenesis in HCC.

Based on the Warburg's proposal, tumor cells have the capacity to produce energy through glycolysis without having a sufficient oxygen supply. Increased aerobic glycolysis is a common characteristic in rapidly proliferating cancer cells[16,17]. Proliferative activity in HCC is closely correlated with glucose metabolism[2]. HCC displays an aerobic glycolytic phenotype, which often correlates with tumor progression and worse clinical outcomes[18,19]. Therefore, a better understanding of the mechanism of glycolysis could be of paramount significance for the development of new therapeutics for HCC treatment. Here we demonstrate that HAT1 promotes the Warburg effect, in part, via changing gene expression correlated with glucose metabolism in HCC cells. Through this regulation, HAT1 could be an ideal molecular target in HCC therapy.

Cisplatin is one of the chemotherapeutic agents for advanced HCC. Cisplatin resistance is a common problem with improving the survival rate for patients with

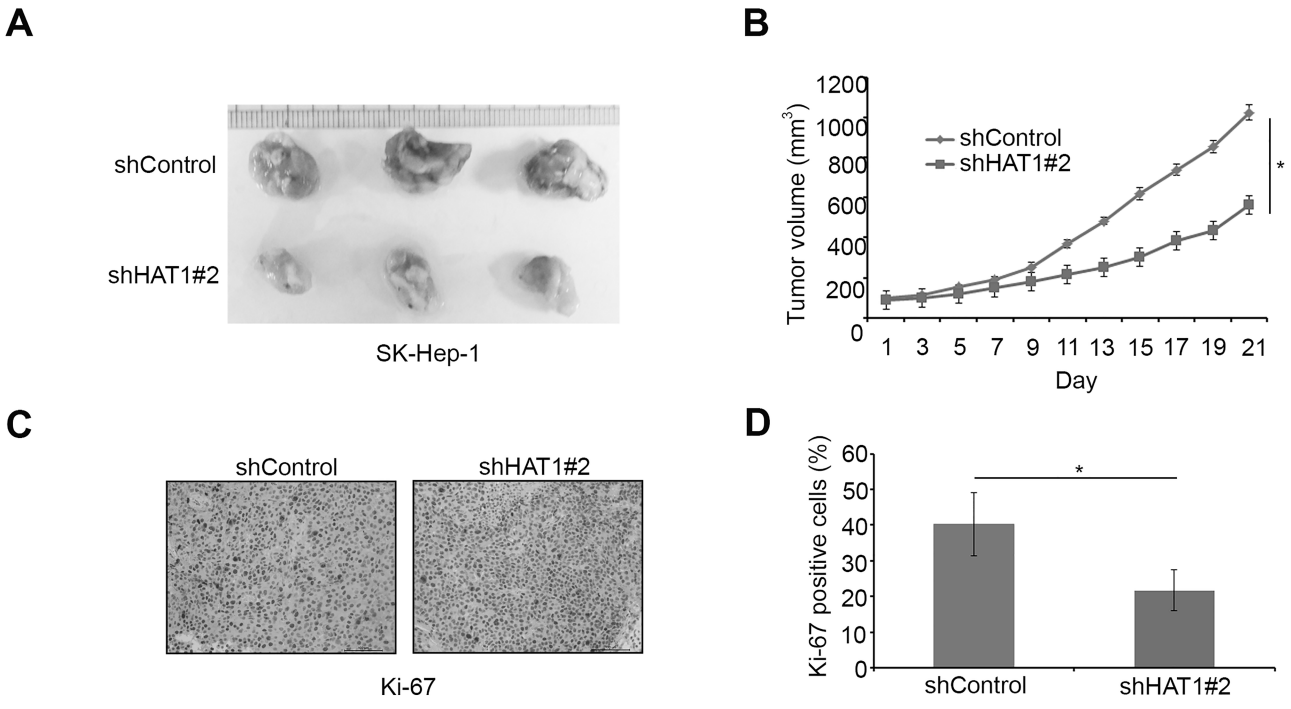

Figure 5. Knockdown of HAT1 inhibits HCC growth in mice. (A, B) SK-Hep-1 cells were infected with control or HAT1-specific shRNAs. Seventy-two hours after infection, cells were injected subcutaneously into the right flank of nude mice. Tumor growth was measured every other day for 21 days, and tumors were harvested and photographed. Data are presented as means ± SD ($n = 3$). *$p < 0.05$. (C, D) IHC analysis of Ki-67 expression in xenografts was performed, and staining was quantified. All data shown are means ± SD (error bar) from 10 replicates. *$p < 0.05$.

HCC[13]. During the development of cisplatin resistance in human cancer cells, numerous epigenetic and/or genetic changes are involved in this process[20]. Here we showed that the knockdown of HAT1 sensitizes HCC cells to apoptotic death induced by cisplatin. It is reported that HAT1 promoted homologous recombination in DNA repair by facilitating histone turnover, and HAT1 plays an important role in the maintenance of genomic stability in higher eukaryotic cells[14]. Meanwhile, our results also showed that HAT1 participated in the aerobic glycolysis process in HCC. Collectively, the abovementioned effect of HAT1 in malignant cells contributes to cisplatin resistance in HCC.

Taken together, our study demonstrated that HAT1 was upregulated in HCC patient specimens and promoted HCC growth in vivo and in vitro. Furthermore, HAT1 regulates glucose metabolism and was involved in cisplatin resistance in HCC cells. These findings have revealed important aspects of the function of HAT1 on the tumor progression and drug resistance in HCC and represent a viable strategy for effective treatment of HCC.

ACKNOWLEDGMENT: This work was supported by the Scientific Research Training Program for Young Talents of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The authors declare no conflicts of interest.

REFERENCES

1. Lafaro KJ, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. Surg Oncol Clin N Am. 2015;24: 1–17.
2. Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol. 2010;7:448–58.
3. Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, Li Y, Robles AI, Chen Y, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat Med. 2003;9:416–23.
4. Zhu RX, Seto WK, Lai CL, Yuen MF. Epidemiology of hepatocellular carcinoma in the Asia-Pacific region. Gut Liver 2016;10:332–9.
5. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol. 2014;28:753–70.
6. Tazi el M, Essadi I, M'Rabti H, Touyar A, Errihani PH. Systemic treatment and targeted therapy in patients with advanced hepatocellular carcinoma. N Am J Med Sci. 2011;3:167–75.
7. Min SK, Koh YH, Park Y, Kim HJ, Seo J, Park HR, Cho SJ, Kim IS. Expression of HAT1 and HDAC1, 2, 3 in diffuse large B-cell lymphomas, peripheral T-cell lymphomas, and NK/T-cell lymphomas. Korean J Pathol. 2012;46: 142–50.
8. Orr JA, Hamilton PW. Histone acetylation and chromatin pattern in cancer. A review. Anal Quant Cytol Histol. 2007;29:17–31.
9. Parthun MR. Hat1: The emerging cellular roles of a type B histone acetyltransferase. Oncogene 2007;26:5319–28.
10. Pogribny IP, Tryndyak VP, Muskhelishvili L, Rusyn I, Ross SA. Methyl deficiency, alterations in global histone modifications, and carcinogenesis. J Nutr. 2007;137: 216S–22S.
11. Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, Thorgeirsson SS, Sun Z, Tang ZY, Qin LX, Wang XW. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer Res. 2010;70:10202–12.
12. 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;55:846–57.
13. McAlpine JA, Lu HT, Wu KC, Knowles SK, Thomson JA. Downregulation of argininosuccinate synthetase is associated with cisplatin resistance in hepatocellular carcinoma cell lines: Implications for PEGylated arginine deiminase combination therapy. BMC Cancer 2014;14:621.
14. Yang X, Li L, Liang J, Shi L, Yang J, Yi X, Zhang D, Han X, Yu N, Shang Y. Histone acetyltransferase 1 promotes homologous recombination in DNA repair by facilitating histone turnover. J Biol Chem. 2013;288:18271–82.
15. Seiden-Long IM, Brown KR, Shih W, Wigle DA, Radulovich N, Jurisica I, Tsao MS. Transcriptional targets of hepatocyte growth factor signaling and Ki-ras oncogene activation in colorectal cancer. Oncogene 2006;25:91–102.
16. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 2009;324:1029–33.
17. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4:891–9.
18. 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;58:229–38.
19. Beyoglu D, Idle JR. The metabolomic window into hepatobiliary disease. J Hepatol. 2013;59:842–58.
20. Shen DW, Pouliot LM, Hall MD, Gottesman MM. Cisplatin resistance: A cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. Pharmacol Rev. 2012;64:706–21.