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# BTF3 promotes proliferation and glycolysis in hepatocellular carcinoma by regulating GLUT1

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**ABSTRACT**

Hepatocellular carcinoma (HCC) is a grievous tumor with an increasing incidence worldwide. Basic transcription factor 3 (BTF3) is discovered to regulate the expression of glucose transporter 1 (GLUT1), which benefits glycolysis, a momentous signature of tumors, through transactivation of the forkhead box M1 (FOXM1) expression. BTF3 is highly expressed in HCC. However, whether BTF3 promotes GLUT1 expression through FOXM1 to modulate glycolysis in HCC remains unclear. The expression profile of BTF3 were determined by online database, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot. The role and mechanism of BTF3 in the proliferation and glycolysis of HCC cells were examined by cell counting kit-8 (CCK-8), 5-ethynyl-2′-deoxyuridine (EdU) incorporation, XF96 Extracellular Flux analyzer, spectrophotometry and western blot analysis. In addition, the direct interaction between BTF3 and FOXM1 was verified by dual-luciferase reporter and co-immunoprecipitation assays. Moreover, the role of BTF3 was also explored in a xenografted mice model. The expression of BTF3 was increased in HCC cells and tumor tissues. Knockdown of BTF3 reduced the cell viability, Edu positive cells, extracellular acidification rate (ECAR), glucose consumption and lactate production in both Huh7 and HCCLM3 cells. The expressions of FOXM1 and GLUT1 were increased in HCC tissues, which were positively correlated with the BTF3 expression. Moreover, a direct interaction existed between BTF3 and FOXM1 in HCC cells. Downregulation of BTF3 decreased the relative protein levels of FOXM1 and GLUT1, which were rescued with overexpression of FOXM1 in both cells. More importantly, overexpression of FOXM1 restored the cell viability, ECAR, glucose consumption and lactate production in both Huh7 and HCCLM3 cells transfected with siBTF3#1. Furthermore, inhibition of BTF3 decreased tumor weight and volume, and the relative level of BTF3, FOXM1, GLUT1 and Ki-67 in tumor tissues from mice xenografted with Huh7 cells. BTF3 enhanced the cell proliferation and glycolysis through FOXM1/GLUT1 axis in HCC.

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## 1. Introduction

Hepatocellular carcinoma (HCC) accounts for about 90% of liver cancer cases, and it is the sixth most common tumors and the fourth leading cause of cancer-related death around the world with an estimation of more than one million cases by 2025.[1,2] There is a wide variety of risk factors of HCC, including hepatitis B, C and D virus infection, alcohol, nonalcoholic steatohepatitis, age, sex and other factors.[3] The current main therapeutic strategies for HCC contains surgical interventions (hepatic resection and liver transplantation), image-guided ablation, radiotherapy, transarterial therapies and systemic therapies.[3,4] Although several approaches, such as surgical resection, liver transplantation, Trans-Arterial Chemoembolization and Radio-Frequency Ablation have been worked on patients with HCC at early stages, the majority of HCC patients are generally diagnosed at advanced stages with the unresectable situation, thereby leading to the poor survival.[5] Moreover, HCC is often accompanied by intrahepatic and extrahepatic metastases, and tumor recurrence and metastasis remain the leading causes of treatment failure for HCC. Therefore, it is an urgent task to clarify the molecular mechanism of HCC and find effective biomarkers and/or therapeutic targets.

Metabolic reprogramming is a important feature of tumor cells, in which tumor cells prefer to break down glucose into lactate via glycolytic pathway due to the energy requirement for fast cell augmentation, also known as the Warburg effect.[6] It has been highlighted to strongly benefit the tumor growth, metastasis, anti-apoptosis and immune escape.[7,8] Numerous studies have demonstrated that glucose metabolism is dysregulated in the initiation and development of HCC.[9] Thus, targeting glucose metabolism-related enzymes, transporters and signaling pathways is a possible and effective strategy for the treatment for HCC. Glucose transporter 1 (GLUT1) encoded by *SLC2A1* gene, is a member of the facilitative glucose transporter family, which can promote the uptake of glucose in various tissues.[10] GLUT1 has been shown to be upregulated in a variety of tumors and is correlated with unfavorable clinical prognosis in patients.[11,12] Consistently, increased expression of GLUT1 is also shown in HCC, which accelerates the progression and glycolysis of HCC.[13,14]

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Basic transcription factor 3 (BTF3) with the molecular weight of 27 KDa also designated as the beta subunit of the nascent polypeptide-related complex is a transcriptional factor that serves a significant role in the promoting of RNA polymerase II.[15] Previous studies has reported that BTF3 participates in the progression of various cancers, such as prostate cancer,[16] osteosarcoma,[17] colorectal cancer,[18] hypopharyngeal squamous cell carcinoma[19] and breast cancer.[20] Particularly, BTF3 is revealed to be upregulated in HCC.[21] More importantly, knockdown of BTF3 significantly reduced the FOXM1 expression in gastric cancer,[22] and FOXM1 has been confirmed to benefit the glycolysis through transactivating the expression of GLUT1 in HCC.[14] However, whether BTF3 promotes GLUT1 expression through FOXM1 to regulate glycolysis in HCC is unclear.

In the current study, the role and mechanism of BTF3 were explored in Huh7 and HCCLM3 cells and mice xenografted with Huh7 cells. The results showed that BTF3 enhanced proliferation and glycolysis by FOXM1/GLUT1 axis in HCC.

## 2. Materials and methods

### 2.1. Expression profile of BTF3 in HCC based on the online databases

The expression profile of BTF3 in liver hepatocellular carcinoma (LIHC) samples and normal tissue samples (para-carcinoma samples) was determined by the TCGA database, including the UALCAN (http://ualcan.path.uab.edu/index.html), the Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) and the GEPIA (http://gepia2.cancer-pku.cn). The survival rate was assessed via the GEPIA database.

### 2.2. Tissue specimen

In total, 30 pairs of HCC tissues and adjacent para-carcinoma tissues were collected from patients with HCC at our Hospital. All the patients without other types of cancers have confirmed by pathological examination and no chemoradiotherapy has been received before surgery. The survival outcomes of BTF3 in the 30 pairs of HCC tissues and adjacent para-carcinoma tissues were determined. The study was confirmed by and Ethics Committee of Harbin Medical University Cancer Hospital, Harbin, and all participants signed the written informed consent (Approval No. 2018–170-R).

### 2.3. Cell culture and transfection

Huh7 (CL-0120) and HCCLM3 (CL-0278) cells were acquired from Procell (Wuhan, China), and cultured in DMEM medium (PM150210, Procell) with 10% fetal bovine serum (FBS, 12103C, Whitehouse Station, NJ, USA) in an incubator at 37°C with 5% $CO_2$. Two small interfering RNAs (siRNAs) against BTF3 and the negative control (NC) were synthesized from GenePharma (Shanghai, China). The targeted sequences for siRNAs and siNC were: GCAGGCACAAGUGCGCAUUTT (siBTF3#1), GCCGAAAGAAGCCUGGGAAUCA (siBTF3#2) and UUCUCCGAACGUGUCACGUTT (siNC). siBTF3#1, siBTF3#2 and siNC were transfected into Huh7 and HCCLM3 cells to downregulate the expression of BTF3 by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The FOXM1 sequences were inserted into pcDNA vector plasmids, which were then transfected into Huh7 and HCCLM3 cells to upregulate the FOXM1 expression by using Lipofectamine 3000 (Invitrogen) based on the previous description.[23] Both cells were harvested after transfection for 48 h for the subsequent analysis.

### 2.4. Reverse transcription-quantitative polymerase chain reaction (RT-Qpcr)

The relative mRNA expressions of BTF3, FOXM1 and GLUT1 were detected by RT-qPCR assays according to the previous report.[24] Total RNA from Huh7 and HCCLM3 cells, or tumor tissues was extracted using TRIzol reagent (9108–1, TaKaRa Biotechnology Co., Ltd., Dalian, China), and then performed for reverse transcription with Bio-Rad ScripTM cDNA Synthesis Kit (1708890, Bio-Rad Laboratories) in keeping with the working instructions. RT-qPCR was carried out in a 20-μl mixture containing 1 μl of the cDNA templates, 10 μl 2×SYBR Green PCR Mastermix (SR1110, Solarbio, Beijing, China), 1 μl of the 10-μM forward and reverse primers and 8 μl DEPC $ddH_2O$ on the Bio-Rad CFX Manager software (Bio-Rad Laboratories, Inc.). The primer sequences were listed in Table 1. The RT-qPCR conditions were 5 min at 94°C, followed by 40 cycles between 94°C for 45 s and 56°C for 40 s, and 72°C for 60 s.

### 2.5. Cell counting kit-8 (CCK-8) assay

5000 Huh7 and HCCLM3 cells were plated into 96-well plates and maintained at 37°C with 5% $CO_2$. Both cells were added with 10 μl CCK-8 reagents (CA1210, Solarbio) and incubated for 2 h at 37°C based on the previous study.[25] The absorbance was detected at 450 nm by a microplate reader from Thermo Fisher Scientific (Waltham, MA, USA).

### 2.6. The 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay

Huh7 and HCCLM3 cells were seeded into 6-well plates with $6 \times 10^5$ cells/well and hatched at 37°C with 5% $CO_2$.

**Table 1.** The primer sequences.

| Gene name | Forward (5′-3′) | Reverse (5′-3′) |
|---|---|---|
| *BTF3* | GCCAGTCTCCTTAAACTAGTCAG | TTTCACCATTACAGGCCATGCT |
| *FOXM1* | AGTCACACCCTAGCCACTGC | ACCATTGCCTTTGTTGTTCC |
| *GLUT1* | TCTGGCATCAACGCTGTCTTC | CGATACCGGAGCCAATGGT |
| *β-actin* | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |

The BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 647 (C0081S, Beyotime, Shanghai, China) was used for the EdU detection in line with the instruction for use. The cell nucleus was re-stained with Hoechst 33,342 (5 μg/mL, Beyotime) and the images were acquired under a fluorescence microscopy (Olympus, Tokyo, Japan).

### 2.7. Detection of extracellular acidification rate (ECAR)

Huh7 and HCCLM3 cells with $1 \times 10^4$ cells were plated in Seahorse XF 96 cell culture microplates. The ECAR was determined on an XF96 Extracellular Flux analyzer (Seahorse Bioscience, Chicopee, MA, USA) with the Seahorse XF-96 Wave software by the Seahorse XF Glycolysis Stress Test Kit (103020–100, Seahorse Bioscience). Glucose, oligomycin, and 2-deoxy-D-glucose (2-DG) were continuously added into each well at 20 min, 40 min and 60 min, respectively. The ECAR values were examined every 6 min. The ECAR value was normalized to the number of cells.

### 2.8. Measurement of glucose consumption and lactate production

After the transfection for 48 h, the culture supernatants were collected for the examination of the levels of glucose and lactate by using a glucose assay kit (CBA086, Sigma, St. Louis, MO, USA) and lactate assay kit (K607, Biovision, Mountain View, CA, USA) according to the operating manual, respectively.

### 2.9. Dual-luciferase reporter assay

Transcription factors are a type of protein molecules with a special structure that exercise the function of regulating gene expression, also known as a trans-acting factor. Some transcription factors bind only to a specific order in their target promoter, and these specific sequences are called cis-factors. The DNA-binding domain of transcription factors and cis-factor bind covalently, a type of binding between protein and DNA, thereby inhibiting or enhancing the expression of genes. Dual-luciferase reporter assays are an important means of detecting specific order binding in these transcription factors and their target promoters. The wild-type sequences of FOXM1 (FOXM1 WT) and its corresponding mutant sequences (FOXM1 MUT) were inserted into pGL3-Basic luciferase vector (2 μg/ml, GenePharma). The binding site sequences of WT-GLUT1 and FOXM1 were ACAAATAA, which were not included in the binding site sequences of MUT-GLUT1 and FOXM1. Huh7 and HCCLM3 cells were seeded into 24-well plates at a density of $4 \times 10^4$ cells/well and maintained at 37°C. When reached about 70% confluency, cells were co-transfected with luciferase reporter plasmids, si-FOXM1 or si-NC, and *Renilla* luciferase pRL-TK plasmid (as the endogenous normalization controls) (100 ng/ml, GenePharma) by Lipofectamine 3000 (Invitrogen) based on the manufacturer's specifications. Luciferase activity was measured with a Dual-Luciferase Reporter Assay kit (E1910, Promega Corporation, Madison, WI, USA) after the transfection for 24 h.

### 2.10. Co-immunoprecipitation (Co-IP) assay

Co-IP is a specific binding of antigens and antibodies, which is widely used to detect protein–protein interactions. This method is often used to identify the presence of an interaction between two proteins of interest, and can also be used to determine the interaction between a known protein and other unknown proteins. Huh7 and HCCLM3 cells were collected and protein was isolated with lysis buffer with protease inhibitors on ice. After 40 min, the supernatant was harvested. BTF3, FOXM1, or immunoglobulin G (IgG) antibody was appended and incubated overnight at 4°C. Protein A/G-agarose beads was added and shaken at 4°C. The pelleted cells were yielded and rinsed with lysis buffer. The precipitate was boiled with loading buffer for 5 min and examined by western blotting.

### 2.11. Animal experiment

BALB/c nude mice with age of 4 weeks old were bought from Cyagen (Jiangsu, China), and raised in a specified pathogen-free (SPF) environment with the 12-h cycle of light-dark and the controlled temperature. All animal experiments were licensed by the Animal Research Ethics Committee of The Fourth Hospital of Harbin Medical University (Approval No.2020-WZYSLLSC-14). Mice were randomly divided into sh-NC group and sh-BTF3 group ($n = 5$). Mice in both groups were subcutaneously inoculated with $1 \times 10^6$ of Huh7 cells[26] transfected with negative control short hairpin RNA (sh-NC) and shRNA targeting BTF3, respectively, as the previous report.[27] The sh-BTF3 (5′-GCAGCGAACACTTTCACCATT-3′) and sh-NC (5′-TTCTCCGAACGTGTCACGT-3′) were transfected into the lentiviral vector pLKO.1-puro (Addgene, Inc., Cambridge, MA, USA). Then, the constructed lentiviral vector plasmids were co-transfected by using Lipofectamine 3000 (Invitrogen) into Huh7 cells with packaging plasmids to form a shBTF3-expressing lentivirus or empty vector lentivirus. Tumor volume was measured every one week for sequential four weeks and calculated as the following formula: volume = 1/2×length×width$^2$. After 28 days, mice were intraperitoneally injected with intraperitoneal injection of 100 mg/kg sodium pentobarbital for sacrifice. The tumors samples were removed, weighed and immobilized in 4% formaldehyde for the immunohistochemistry assay.

### 2.12. Immunohistochemistry(IHC)

The fixed tumor tissues were dehydrated with ethanol, implanted into paraffin (YA0011, Solarbio) and cut into sections with the thickness of 5 μm. After restored with sodium citrate buffer (pH 6.0, P0081, Beyotime) at 94°C for 15 min, sections were treated with 1% bovine serum albumin (BSA, ST2249, Beyotime) for 1h and hatched with primary antibodies, including anti-BTF3 (1:250, ab203517, Abcam), anti-FOXM1 (1:250, ab207298, Abcam), anti-GLUT1 (1:100, ab128033, Abcam) and

anti-Ki-67 (1:1000, ab15580, Abcam, Cambridge, UK), and HRP labeled anti-rabbit IgG antibody (ab288151, Abcam). Finally, sections were re-stained with hematoxylin, and photographed using a light microscope (Olympus).

### 2.13. Western blot

Western blot assays were performed as the previous methods.[28,29] Total proteins were prepared from Huh7 and HCCLM3 cells, as well as tumors samples through the introduction with RIPA lysis buffer (P0013B, Beyotime) and quantified with BCA kit (P0012S, Beyotime) in accordance with the operation instruction. 20 μg protein samples were dissolved with 10% SDS-PAGE, and electrically shift onto PVDF membranes for the conventional operations of western blot experiment. The primary and second antibodies included anti-BTF3 (1:1000, ab203517, Abcam, Cambridge, UK), anti-FOXM1 (1:2000, ab180710, Abcam), anti-GLUT1 (1:1000, ab128033, Abcam), anti-β-actin (1:1000, ab8227, Abcam) and goat anti-rabbit IgG H&L (HRP) (ab6721, 1:10000, Abcam). The membranes were developed with ECL Western Blotting Detection Kit (Goat IgG) (SW2030, Solarbio), and the band intensity was determined by ImageJ software (National Institutes of Health, USA).

### 2.14. Statistical analysis

All results were expressed as the form of mean ± standard deviation (SD). The statistical differences of all the data were performed by the Student's *t*-test (two groups) or the one-way analysis of variance (ANOVA) (≥ two groups) followed by *Post Hoc* Bonferroni test via SPSS 26.0 software (IBM, Armonk, New York, USA). Significant difference was defined when $P < .05$.

## 3. Results

### 3.1. BTF3 was highly expressed in HCC

To explore the role of BTF3 in HCC, the expression of BTF3 was first detected in HCC. As shown in Figure 1a–d, a significant increase in the expression of BTF3 was observed in HCC samples compared with para-carcinoma samples,

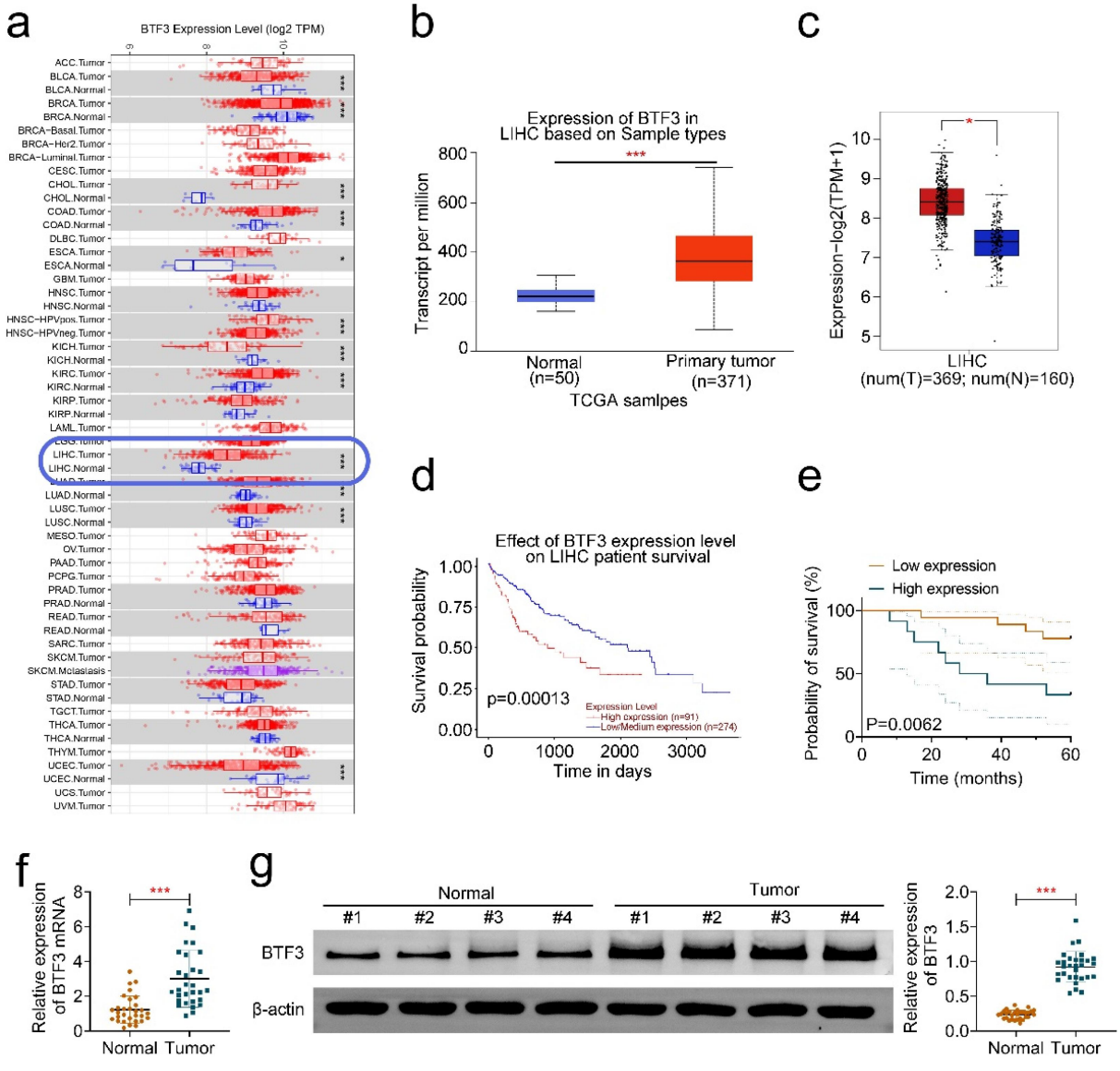


**Figure 1.** The BTF3 level was upregulated in HCC.

which was further predicted the poor survival possibility in patients with HCC according to the online database. Moreover, high expression of BTF3 predicted the poor survival possibility in patients with HCC from clinical surgery (Figure 1e). Besides, the transcriptional and translational expressions of BTF3 were consistently upregulated in the tumor tissues from patients with HCC relative compared with para-carcinoma tissues (Figure 1f,g). Therefore, the expression level of BTF3 was enhanced in HCC.

### 3.2. Knockdown of BTF3 inhibited the HCC cell proliferation

Since the level of BTF3 was elevated in HCC, two siRNAs targeting to BTF3 (siBTF3#1 and siBTF3#2) were transfected into Huh7 and HCCLM3 cells to downregulate the expression of BTF3 in HCC cells. Both siBTF3#1 and siBTF3#2 prominently reduced the relative mRNA and protein expression of BTF3 in Huh7 and HCCLM3 cells, indicating a practicable transfection efficiency in HCC cells (Figure 2a,b). Inhibition of BTF3 significantly decreased the cell viability and Edu positive cells in both Huh7 and HCCLM3 cells compared with these in cells transfected with si-NC and in cells without transfection (Figure 2c,d). Thus, silencing of BTF3 suppressed the proliferation of HCC cells.

### 3.3. Inhibition BTF3 repressed glycolysis in HCC cells

Then, the role of BTF3 in glycolysis was assessed in both Huh7 and HCCLM3 cells. Results from Figure 3a showed that the ECAR was observably diminished in Huh7 and HCCLM3 cells transfected with siBTF3#1 and siBTF3#2 compared with cells

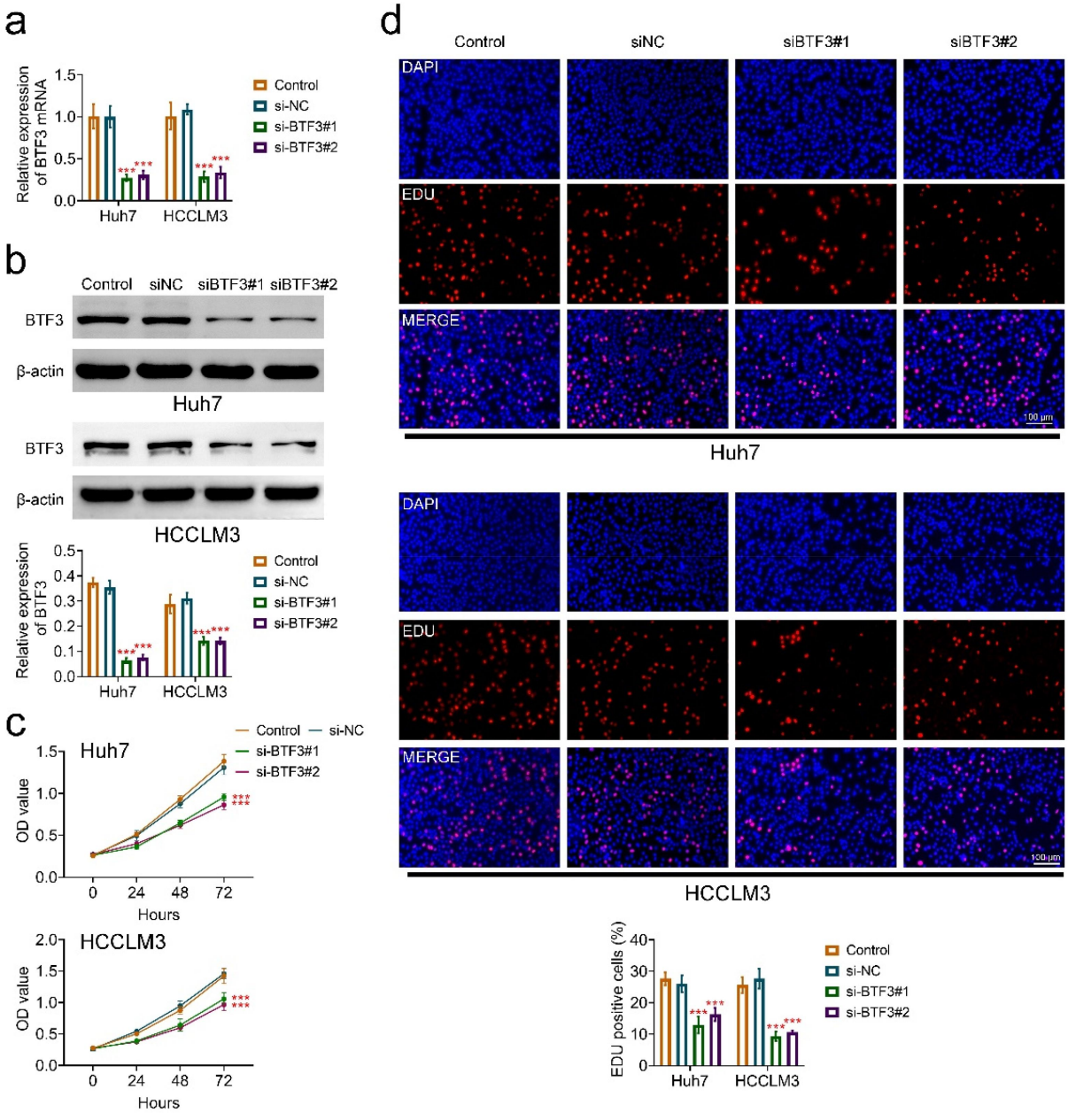


**Figure 2.** Downregulation of BTF3 restrained the proliferation of Huh7 and HCCLM3 cells.

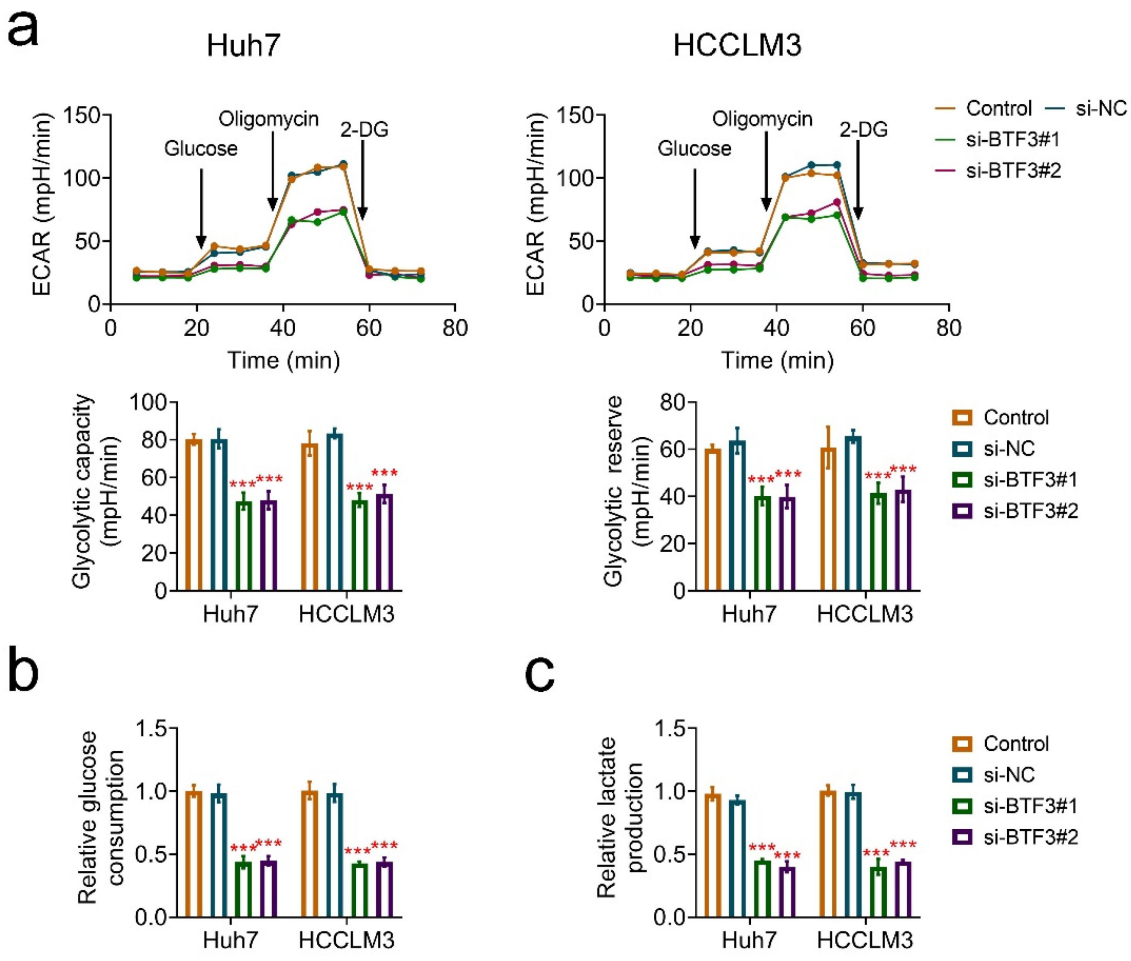


**Figure 3.** Downregulation of BTF3 limited glycolysis in both Huh7 and HCCLM3 cells.

transfected with si-NC and cells without transfection. Meanwhile, knockdown of BTF3 markedly declined the glucose consumption and lactate production compared with the si-NC group and control group (Figure 3b,c). Hence, silencing of BTF3 restricted glycolysis in both Huh7 and HCCLM3 cells.

### 3.4. BTF3 promoted the GLUT1 expression via FOXM1

BTF3 has been revealed to modulate the FOXM1 expression, which benefit glycolysis through transactivating the GLUT1 expression. Our results showed that the relative mRNA expressions of FOXM1 and GLUT1 were significantly increased in the tumor tissues from patients with HCC compared with para-carcinoma samples (Figure 4a,b). Thus, a markedly positive relationship was observed between the BTF3 expression and the FOXM1 expression, and the BTF3 expression and the GLUT1 expression (Figure 4c,d). Moreover, the direct interaction between BTF3 protein and FOXM1 protein was also confirmed by Co-IP results in HCC cells (Figure 4e). Besides, the interaction between FOXM1 and GLUT1 was explored by Dual-luciferase reporter assays (Figure 4f). The results showed that co-transfection of GLUT1-WT and si-FOXM1 was observably reduced the relative fluorescence intensity compared with co-transfection of GLUT1-WT and si-NC in both Huh7 and HCCLM3 cells. However, no statistical difference was discovered in the relative fluorescence intensity after both Huh7 and HCCLM3 cells were co-transfected with GLUT1-MUT and si- FOXM1 compared with co-transfection of FOXM1-MUT and si-NC (Figure 4f). Therefore, a direct interaction existed between GLUT1 and FOXM1 in HCC cells. Moreover, the expressions of FOXM1 and GLUT1 in both Huh7 and HCCLM3 cells were detected after both cells were transfected with siBTF3#1 and siBTF3#2. Knockdown of BTF3 notably reduced the relative protein expression of FOXM1 and GLUT1 in both Huh7 and HCCLM3 cells compared with those in cells transfected with si-NC and in cells without transfection (Figure 4g). To further confirm the relation between BTF3 and FOXM1/GLUT1 axis, FOXM1 was overexpressed in both Huh7 and HCCLM3 cells. Overexpression of FOXM1 significantly rescued the relative protein expression of FOXM1 and GLUT1 in both Huh7 and HCCLM3 cells transfected with siBTF3#1 (Figure 4h). Thus, BTF3 increased the GLUT1 expression via FOXM1.

### 3.5. BTF3 enhanced proliferation and glycolysis of HCC cells via GLUT1

Moreover, knockdown of BTF3 markedly reduced the cell viability in Huh7 and HCCLM3 cells, which were obviously restored with the overexpression of FOXM1 (Figure 5a).

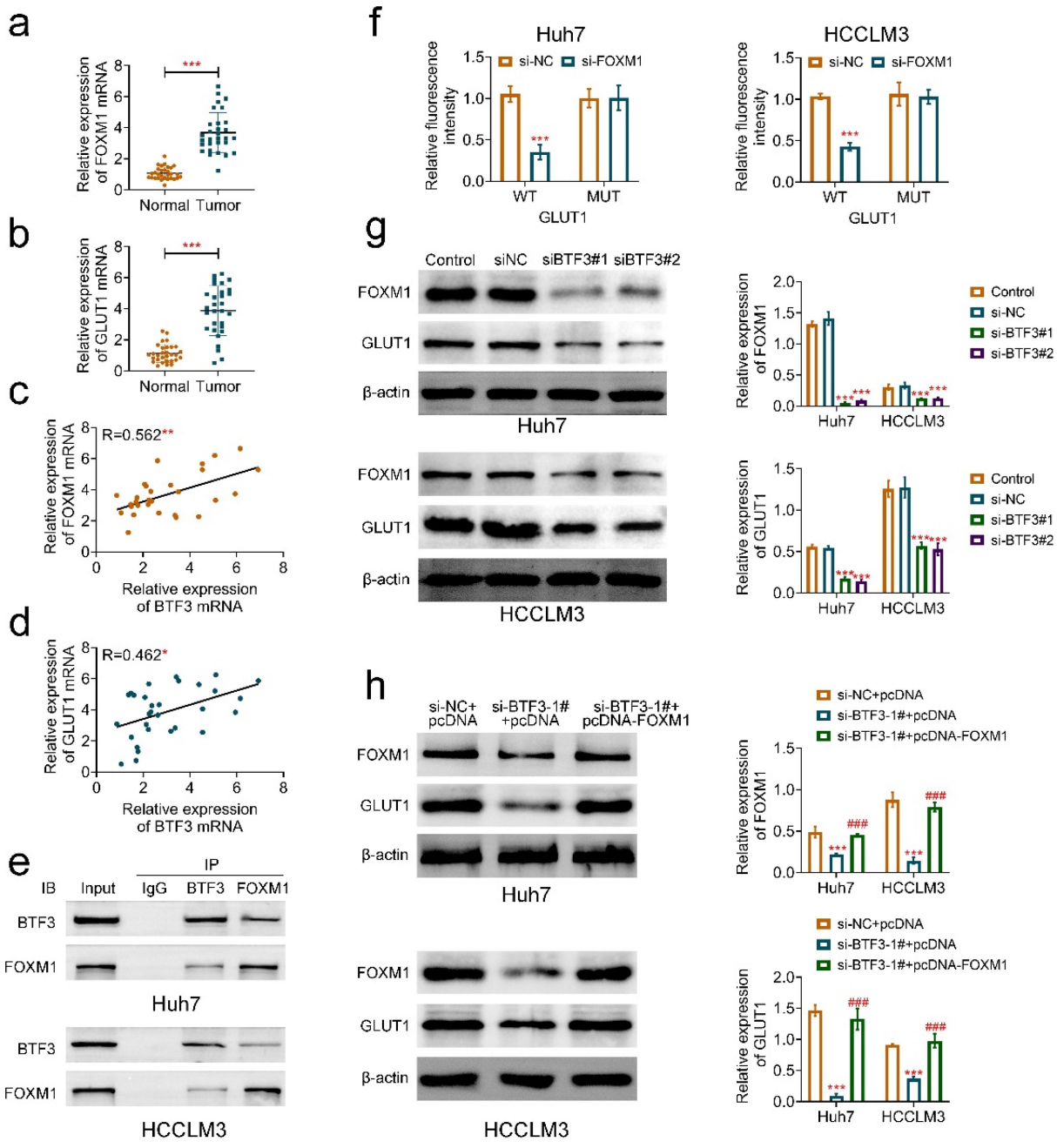


**Figure 4.** BTF3 promoted the GLUT1 expression via FOXM1.

Similarly, overexpression of FOXM1 significantly recovered the decreased ECAR, glucose consumption and lactate production in Huh7 and HCCLM3 cells transfected with siBTF3#1 (Figure 5b,c). Collectively, BTF3 increased proliferation and glycolysis of HCC cells via FOXM1/GLUT1 axis.

### 3.6. Silencing of BTF3 impeded HCC tumor growth in vivo

Furthermore, mice xenografted with Huh7 cells transfected with sh-BTF3 exhibited a reduced tumor weight and tumor volume compared with those injected with Huh7 cells transfected with sh-NC (Figure 6a). In addition, inhibition of BTF3 also obviously decreased the relative level of BTF3, FOXM1, GLUT1 and Ki-67 in tumor tissues from mice xenografted with Huh7 cells (Figure 6b). Moreover, the relative protein levels of FOXM1 and GLUT1 were notably reduced in tumor tissues from mice xenografted with Huh7 cells (Figure 6c). Therefore, knockdown of BTF3 inhibited HCC tumor growth in mice.

## 4. Discussion

The role and mechanism of BTF3 in HCC were investigated *in vitro* and *in vivo* in the current study. The expression of BTF3 was increased in HCC based on the results from online databases and in tumor tissues from patients with HCC. Knockdown of BTF3 reduced the cell viability, Edu positive cells, ECAR, glucose consumption and lactate production in

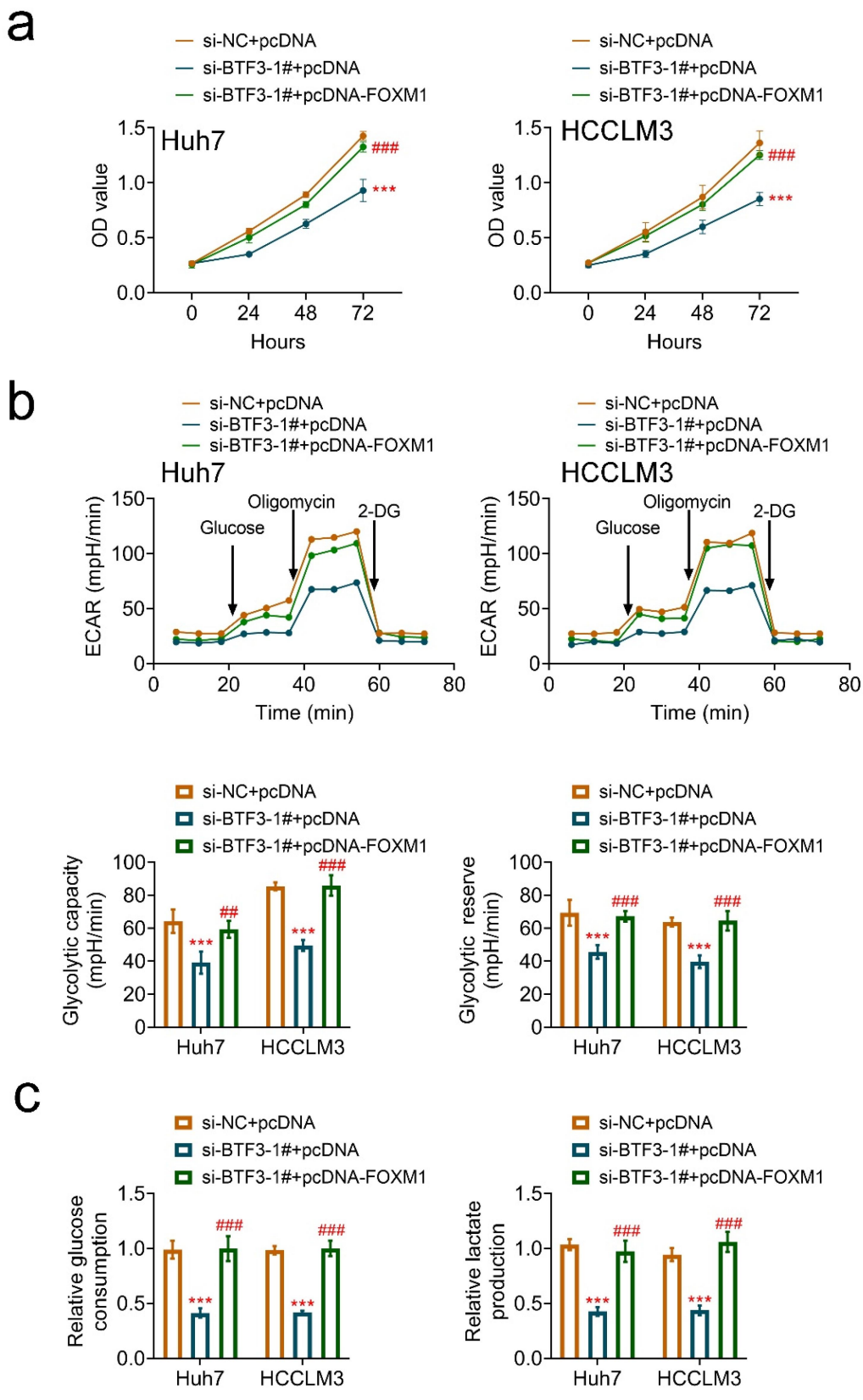


**Figure 5.** BTF3 accelerated proliferation and glycolysis of HCC cells via FOXM1/GLUT1 axis.

both Huh7 and HCCLM3 cells. Moreover, a direct interaction existed between BTF3 and FOXM1 in HCC cells. Downregulation of BTF3 decreased the relative protein levels of FOXM1 and GLUT1, which were rescued with overexpression of FOXM1 in both Huh7 and HCCLM3 cells. More importantly, overexpression of FOXM1 restored the cell viability, ECAR, glucose consumption and lactate production in both Huh7 and HCCLM3 cells transfected with siBTF3#1.

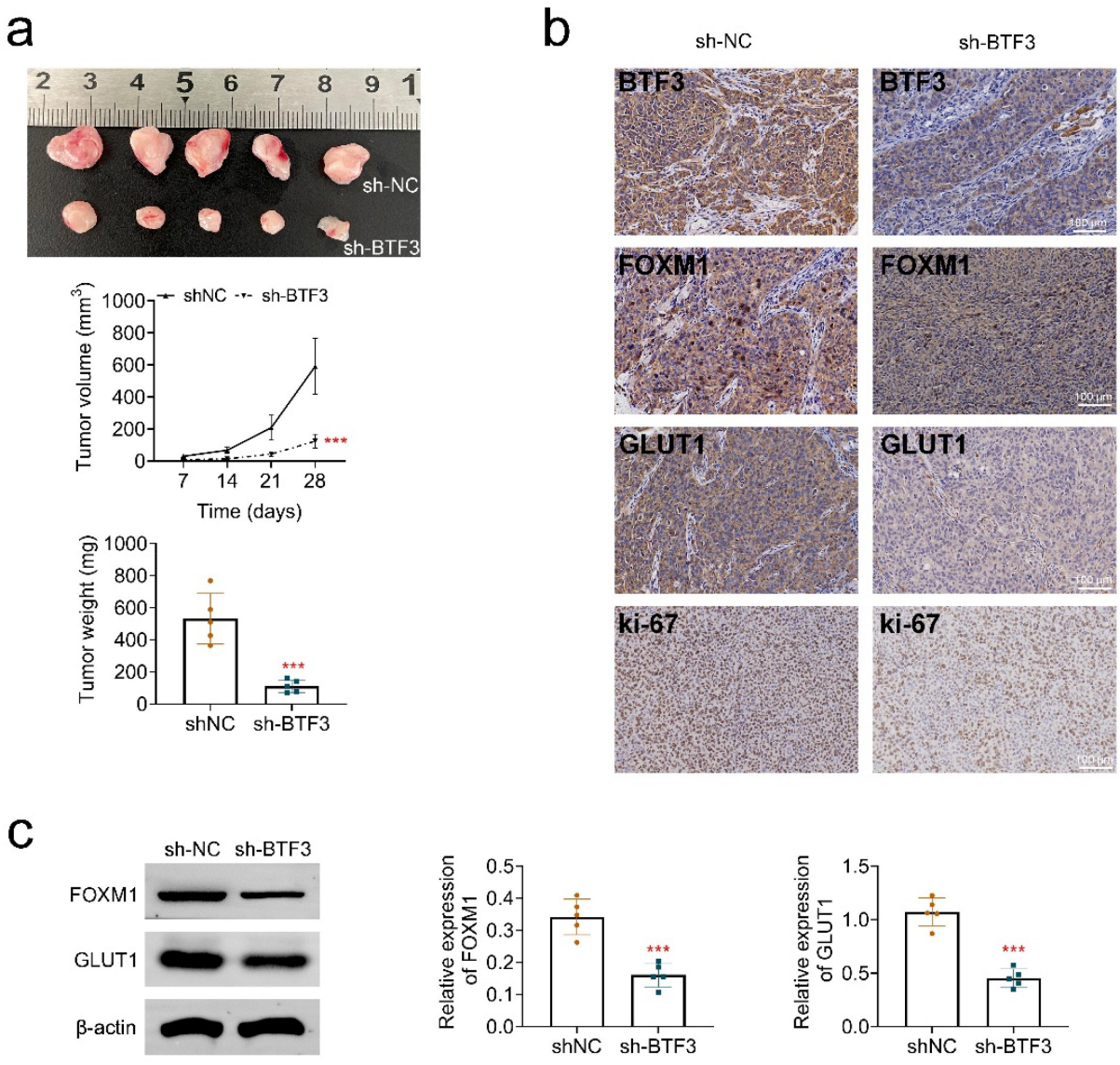


**Figure 6.** Inhibition of BTF3 repressed HCC cell growth in mice.

Furthermore, inhibition of BTF3 reduced tumor weight and volume, and the relative levels of BTF3, FOXM1, GLUT1 and Ki-67 in tumor tissues from mice xenografted with Huh7 cells. Collectively, BTF3 contributed to proliferation and glycolysis through FOXM1/GLUT1 axis in HCC.

Increasing evidence suggests that BTF3 may be a potential therapeutic target in a variety of tumors due to its dysregulation, such as upregulation in prostate cancer,[16,30] osteosarcoma,[17] colorectal cancer[18,31] and hypopharyngeal squamous cell carcinoma.[19] In line with these findings, the expression of BTF3 was also enhanced in HCC based on the results from online databases and in tumor tissues from patients with HCC in our study. Moreover, upregulation of BTF3 predicted a poor survival possibility in patients with HCC, which was similar to the results in colorectal cancer,[18] hypopharyngeal squamous cell carcinoma[19] and gliomas.[32] Therefore, BTF3 might serve as a biomarker for HCC diagnosis and a therapeutic target for the treatment of HCC.

Proliferation is a well-recognized hallmark of tumors, which exerts a critical role in the progression of cancer.[33] Inhibition of BTF3 suppresses the osteosarcoma cells proliferation.[17] BTF3 silencing inhibited cell proliferation and enhances cell apoptosis and cell cycle in hypopharyngeal squamous cell carcinoma.[19] Additionally, the inhibitory role of BTF3 in proliferation is also observed in triple-negative breast cancer,[20] prostate cancer,[16] and gastric cancer.[22] In the present study, knockdown of BTF3 reduced the cell viability and Edu positive cells in both Huh7 and HCCLM3 cells. Significantly, inhibition of BTF3 reduced tumor weight and volume, and the relative level of Ki-67 in tumor tissues from mice xenografted with Huh7 cells. Ki-67 is closely associated with cell division,[34] and thus, Ki-67 is a standard marker of proliferation that are widely applied to assess the growth ratio of a cell population.[35] Taken together, downregulation of BTF3 inhibited HCC cell growth both *in vitro* and *in vivo*.

Metabolic reprogramming has gained more and more attention as one of the significant features of tumor cells.[33] Glycolysis has been shown to be closely related to the development of HCC.[36] Thus, a series of approaches targeting glycolysis has been demonstrated to be potential strategies for the treatment of HCC. For instance, fatty acid receptor CD36 mediates aerobic glycolysis that promotes proliferation

and metastasis of HCC.[37] Histone deacetylase 11 (HDAC11) regulates progression, resistance and stemness through modulating glycolysis in HCC.[38] Glycogen synthase kinase-3 beta (GSK-3β) promotes growth, metastasis and tumorigenicity of HCC via enhancing glycolysis.[39] Here, our results showed that knockdown of BTF3 reduced the ECAR, glucose consumption and lactate production in both Huh7 and HCCLM3 cells. ECAR is a direct indicator for the quantification glycolysis.[40] Thus, inhibition of BTF3 repressed glycolysis in HCC.

FOXM1 belongs to the FOX superfamily and is a representative transcription factor related to proliferation and cell fate decisions.[41] As a core gene of HCC,[42] FOXM1 has been highlighted to participate in the progression of HCC, including proliferation, apoptosis, migration, invasion, metastasis and resistance.[43,44] In addition, FOXM1 is also involved in the immune infiltration[45] and predicts the prognosis of HCC.[46] More importantly, FOXM1 transactivates the expression of GLUT1 to modulate glycolysis in HCC.[14,47] In the present study, a prominent increase in the relative mRNA expressions of FOXM1 and GLUT1 were indicated in the tumor tissues from patients with HCC. Thus, a markedly positive relationship was observed between the BTF3 expression and the FOXM1 expression, and the BTF3 expression and the GLUT1 expression. Moreover, a direct interaction was discovered between BTF3 and FOXM1 in HCC cells. Downregulation of BTF3 decreased the relative protein levels of FOXM1 and GLUT1, which were rescued by overexpression of FOXM1, indicating that BTF3 promoted the GLUT1 expression via FOXM1 in HCC. More importantly, overexpression of FOXM1 restored the cell viability, ECAR, glucose consumption and lactate production in both Huh7 and HCCLM3 cells transfected with siBTF3#1. Collectively, BTF3 promoted proliferation and glycolysis of HCC cells via FOXM1/GLUT1 axis.

In summary, BTF3 was upregulated in HCC. Knockdown of BTF3 inhibited proliferation and glycolysis of HCC via FOXM1/GLUT1 axis. However, the direct role of BTF3/FOXM1/GLUT1 axis in mice with HCC should be explored in the following study. In addition, the clinical characteristics of BTF3 will be analyzed by collecting the clinical patient data in the future studies. In conclusion, our data illustrate that BTF3 has the potential to serve as a biomarker for the diagnosis and therapy of HCC.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

All data generated or analyzed during this study are included in this published article.
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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## Author contributions

Peng Wang and Jianmin Sun designed the study, completed the experiment and supervised the data collection, Chengming Sun analyzed the data, interpreted the data, Haoran Zhao, YuBao Zhang and Jing Chen prepare the manuscript for publication, and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

## Ethics approval

All procedures performed in studies involving human participants were in accordance with the standards upheld by the Ethics Committee of Harbin Medical University Cancer Hospital, Harbin and with those of the 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects (Approval No. 2018–170-R).

All animal experiments were approved by the Ethics Committee of The Fourth Hospital of Harbin Medical University for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines (Approval No.2020-WZYSLLSC-14).

## Statement of informed consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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