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NUDT5 promotes the growth, metastasis, and Warburg effect of IDH wild-type glioblastoma multiforme cells by upregulating TRIM47

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ABSTRACT

Objective: To explore the regulatory mechanism of NUDT5 in glioblastoma multiforme (GBM).

Methods: GEPIA database was used to predict the expressions of NUDT5 and tripartite motif family proteins 47 (TRIM47) in GBM patients. RT-qPCR and Western blot analyses were performed to examine NUDT5 expression in GBM cells. LN-229 cell proliferation, migration as well as invasion were estimated by CCK-8, colony formation, wound healing, and Transwell assays following interference with NUDT5. ECAR assay, *L*-lactic acid kit, glucose detection kit, and ATP detection kit were applied for the detection of glycolysis-related indexes. Co-immunoprecipitation experiment was carried out to verify the relationship between NUDT5 and TRIM47.

Results: GEPIA database showed that NUDT5 expression was significantly increased in GBM patients. Inhibiting the expression of NUDT5 in GBM cells significantly suppressed the viability, proliferation, invasion, migration, and glycolysis of GBM cells. Moreover, TRIM47 was highly expressed in GBM cells and interacted with NUDT5. Overexpression of TRIM47 partially reversed the inhibitory effect of NUDT5 downregulation on the proliferation, metastasis, and glycolysis of GBM cells.

Conclusions: NUDT5 promotes the growth, metastasis, and Warburg effect of GBM cells by upregulating TRIM47. Both NUDT5 and TRIM47 can be used as targets for GMB treatment.

KEYWORDS: Glioblastoma multiforme; NUDT5; TRIM47; Growth; Metastasis; Warburg effect

1. Introduction

Glioblastoma multiforme (GBM) can be divided into isocitrate dehydrogenase (IDH) wild type and IDH mutant. The clinical outcome of patients with IDH wild-type GMB is poorer than that with IDH mutant GMB[1]. IDH wild-type GMB frequently involves O6-methylguanine-DNA methyltransferase promoter methylation and *ATRX*, *TERT*, and *BRAF* gene mutation[2]. In addition, *connexin 43* mRNA levels are elevated in high-grade GBM, the mutations and expression of which can be used as diagnostic criteria for GBM[3]. The median survival of GBM patients is about 4-10 months, and the prognosis of multiple GBM is worse than that of single GBM[4]. Therefore, finding new therapeutic targets and exploring the pathogenesis of GBM is the top priority of current research.

Significance

NUDT5 is highly expressed in GBM and can be used as an independent prognostic factor of GBM. However, the specific regulatory role of NUDT5 in GBM has not been reported so far. This study shows that NUDT5 promotes the growth, metastasis, and Warburg effect of GBM cells by upregulating TRIM47.

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Nudix hydrolase 5 (NUDT5), which is a mutant-related protein, is involved in the key processes of nucleotide metabolism and cancer development. It was reported that NUDT5 level was positively correlated with the prognosis of clear cell renal carcinoma[5]. Besides, RNA oxidation level, cell senescence, and apoptosis rate were increased and cell viability was decreased in the fibroblast IMR-90 cells[6]. It has been confirmed that NUDT5 downregulation significantly delayed cell cycle during the G_1 phase in cervical cancer HeLa cells, and abolished S and G_2/M phase cell cycle arrest to impede cell proliferation[7]. The high expression of MTH1 and NUDT5 predicted the low survival rate of esophageal squamous cell carcinoma and was associated with the malignancy of esophageal squamous cell carcinoma[8]. It was noted that NUDT5 displayed ascending expression in GBM patients based on the Cancer Genome Atlas (TCGA) database[9]. Nonetheless, NUDT5 expression in GBM cells and its regulatory mechanism have not been reported so far.

The GeneMANIA database predicted the potential interaction between NUDT5 and tripartite motif family proteins 47 (TRIM47). TRIM47 is an important member of TRIM family. TRIM47 has been reported to be implicated in the progression of diverse malignancies[10–12]. A previous study has shown that *TRIM47* gene knockout inhibited the aggressive phenotypes of glioma cells through inactivation of Wnt/beta-catenin pathway[13]. TRIM47 mediated FBP1 ubiquitination to facilitate aerobic glycolysis and development of pancreatic cancer[14]. *TRIM47* gene knockout inhibited the occurrence and progression of breast cancer by inactivating the PI3K/Akt pathway[15]. However, the potential interaction between NUDT5 and TRIM47 in GBM is still vague. Therefore, in this paper, we aimed to investigate the expressions of NUDT5 and TRIM47 in GBM cells and their underlying mechanisms.

2. Materials and methods

2.1. Database

GEPIA (hppt//:www.gepia.cancer-pku.cn)[16] database was used to predict the expressions of NUDT5 and TRIM47 in GBM and the correlation between TRIM47 expression and the overall survival rate of GBM patients. In addition, The GeneMANIA (http://genemania.org/)[17] and GEPIA databases were applied to predict the potential relationship between NUDT5 and TRIM47 in GBM.

2.2. Cell culture

DMEM (Gibco) supplemented with 10% fetal bovine serum that was placed in a humidified environment at 37℃ with 5% CO_2 was applied for the cultivation of normal human astrocytes (NHA cells, SVG p12 cells, cat. no. CRL-8621, ATCC, USA) and astrocytoma cell lines U-251MG (cat. no. BNCC101001, BeNa Culture Collection, China) and SHG-44 (cat. no. ml057238, Mlbio, China), GBM cell lines BT-325 (cat. no. ml055607, Mlbio, China) and LN-229 (cat. no. BNCC341218, BeNa Culture Collection, China). After the recovery of all the above cells, the cells were cultured normally and used for further experiments after 3 passages.

2.3. Quantitative real–time PCR (RT–qPCR)

Total cellular RNA was extracted using RNAiso Plus (TaKaRa, Shiga, Japan). cDNA was produced from the RNA specimens using Primescript RT Reagent Kit (TaKaRa). The RT conditions were as follows: 37℃ for 15 min, 85℃ for 5 s and 4℃. According to the manufacturer's instructions, PCR amplification was conducted using an SYBR Premix DimerEraser kit (TaKaRa) in ABI 7600 system (Applied Biosystems, Foster, CA, USA). The thermocycling conditions were as follows: predenaturation at 95℃ for 5 min, followed by 40 cycles consisting of a 10 s denaturing step at 95℃, a 10 s annealing step at 60℃ and a 20 s extension step at 72℃. The relative gene expression was normalized against *GAPDH* using the $2^{-\Delta\Delta Ct}$ method[18] (Table 1).

2.4. Western blot

Total proteins extracted from sample LN-229 cells with a RIPA lysis buffer (Biosharp) were quantified using a bicinchoninic acid protein assay kit (Biosharp). PVDF membranes (Thermo, USA) were used to transfer 30 μg of protein that was uploaded into each lane and solubilized in 10% SDS-PAGE Gel. The primary antibodies including NUDT5 (1∶1 000, ab129172, Abcam), Ki-67 (1∶1 000, ab16667, Abcam), PCNA (1∶1 000, ab29, Abcam), E-cadherin (1∶1 000, ab40772, Abcam), *N*-cadherin (1∶1 000, ab245117, Abcam), vimentin (1∶1 000, ab92547, Abcam), glucose transporter type 1 (GLUT1, 1∶1 000, ab115730, Abcam), hexokinase 2 (HK2, 1∶1 000, ab209847, Abcam), TRIM47 (1∶1 000, ab72234, Abcam), and GAPDH (1∶1 000, ab8245, Abcam) and anti-rabbit IgG secondary antibody (1∶1 000; Abcam) were supplemented to the PVDF membranes for incubation. The observation of protein bands was performed with Enhanced ECL Chemiluminescent Substrate Kit [Yeasen Biotechnology (Shanghai) Co., Ltd.], and the ChemiDoc imaging system (Bio-Rad Laboratories, Inc) was applied for the quantification.

Table 1. Primer sequences used in RT-qPCR analysis.

Genes	Forward	Reverse
NUDT5	CAAGAACCAACGGAAT CTTCTCA	TGTTTCACCAGAACGA TACACTC
TRIM47	CTGAGCAGTCCAAAGT CCTGA	CTACGGCTGCACTCTT GATG
GAPDH	AATGGGCAGCCGTTAGGAAA	GCGCCCAATACGACCA AATC

2.5. Cell transfection

The overexpression plasmids specific to TRIM47 (Ov-TRIM47) and empty vector (Ov-NC) with a lentiviral expression vector GV 493 were synthesized by Shanghai GenePharma Co., Ltd., and short hairpin RNA targeting NUDT5 (sh-NUDT5#1/2) and the corresponding negative control (sh-NC) were provided by BioVector NTCC, Inc. According to the manufacturer's instructions, the recombinants (20 nM) were delivered into LN-229 cells (10^7 cells) employing Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.). The transfection efficiency was tested 48 h post-transfection. The target sequence of sh-NUDT5#1 is GGTGGTATGGAATTGTAATTA, sh-NUDT5#2 GTTGTGACCTTCCTTTAAATT, and sh-NC TTCTCCGAACGTGTCACGT.

2.6. Cell Counting Kit-8 (CCK-8) assay

After the indicated cultivation of LN-229 cells inoculated in 96-well plates (5×10^3 cells/well), OD_{450} value was detected under a microplate reader (TECAN, Grodig, Austria) following the supplementation of 10 μL CCK-8 reagent (Dojindo Molecular Technologies, Tokyo, Japan) at 24, 48 and 72 h respectively at 37℃ for 4 h.

2.7. Colony formation assay

Following 2 weeks of incubation of LN-229 cells inoculated in 6-well plates (500 cells/per well), 100% methanol and 0.5% crystal violet solution (Solarbio, Beijing, China) were added to immobilize and stain cell colonies for 10 min and 30 min at room temperature, respectively. Colonies were then counted and observed by an inverted microscope (Olympus).

2.8. Wound healing assay

After indicated transfection, LN-229 cells (5×10^4 cells/well) were seeded into 96-well plates and incubated until cells have reached 90% confluence. Scratches were made by a 96-wounding replicator (VP Scientific, San Diego, CA, USA) across the cell layer. At 0 and 24 h, a fluorescence microscope was used for photographing and the cell migration rate of each group was calculated.

2.9. Transwell assay

The upper chamber was loaded with LN-229 cells suspended in a 100 μL fetal bovine serum-free medium. Approximately a 600 μL medium supplemented with 30% fetal bovine serum was added into the bottom chamber. After 24 h of incubation, the bottom chamber cells were immobilized by 4% formaldehyde and stained by Giemsa. Finally, five random fields were selected for observation and the invasive ability of cells was analyzed.

2.10. Extracellular acidification rate (ECAR) assay

Following the cultivation of LN-229 cells (4×10^4 cells/well) inoculated in 96-well plates, cells were washed with Seahorse buffer. 10 mM glucose, 1 mM oligomycin, and 80 mM 2-deoxy-*D*-glucose were added to measure the ECAR. The data were analyzed to cell numbers and plotted as ECAR (mpH/min) as a function of time using an XF-96p analyzer.

2.11. Lactate dehydrogenase (LDH) assay

LDH level was tested with an LDH Release Assay Kit (Beyotime). Briefly, the transfected LN-229 cells were exposed to LDH release reagent 1 h before the end of the incubation. Then, cell supernatant was collected and incubated with an LDH detection mixture for 30 min in the dark. OD_{490} value was detected under a SYNERGY HTX multimode reader.

2.12. Glucose detection

LN-229 cells were seeded into plates and a Glucose Assay kit (Sigma, Burlington, MA, USA) was to estimate the glucose consumption following the manufacturer's instructions. Data were analyzed according to the standard curve line and OD value.

2.13. ATP assay

After the cultivation of transfected LN-229 cells with ATP assay lysis buffer, BCA Protein Assay kit (Beyotime, China, catalog no. P0010) was used to test the protein content in the harvested supernatant. Then a 100 μL detection reagent was mixed with 100 μL supernatant, and firefly luciferase activity was detected and analyzed by luminescence spectrometry (EnSpire, USA). Finally, the ATP level was expressed as the percentage relative to that of the control group.

2.14. Co-immunoprecipitation (CO-IP)

After LN-229 cells were lysed in Tris/HCl (pH 7.5) and 1% Triton, the supernatant was incubated with NUDT5 and TRIM47 antibodies (2 μg) for 1.5 h at 4℃. Then, protein A/G-Sepharose beads (GE Healthcare) were added to the samples for another 1.5 h. Laemmli buffer was added to the PBS-rinsed beads for 5 min of incubation at 95℃, and Western blot was finally applied for detection.

2.15. Statistical analysis

The data are expressed as mean±SD and analyzed by Student's *t*-test or ANOVA followed by Tukey's *post hoc* test using Prism 6 (GraphPad Software, Inc., USA). $P<0.05$ was considered statistically significant.

3. Results

3.1. NUDT5 is overexpressed in multiple GBM

GEPIA database showed that NUDT5 expression was abnormally elevated in the tissues of GBM patients (Figure 1A). RT-qPCR and Western blot manifested that NUDT5 mRNA and protein expressions in GBM cells were higher than those in NHA cells (Figure 1B and C). It was noted that NUDT5 had the highest expression in LN-229 cells compared with U-251MG, SHG-44, and BT-325 cells. Therefore, LN-229 cells were used for further study.

3.2. Interference with NUDT5 inhibits GBM cell proliferation

Following the transfection of NUDT5 interference plasmids, RT-qPCR, and Western blot analyses showed that NUDT5 mRNA and protein expressions in LN-229 cells were distinctly depleted by sh-NUDT5 relative to the sh-NC group (Figure 2A and B). NUDT5 had lower expression in the sh-NUDT5#2 group compared with the sh-NUDT5#1 group ($P<0.001$), therefore, sh-NUDT5#2 was used for further experiment. In CCK-8 and colony formation assays, LN-229 cell viability and proliferation were prominently diminished by sh-NUDT5 compared with the sh-NC group ($P<0.001$) (Figure 2C and D). As shown in Figure 2E and F, silencing of NUDT5 markedly obstructed LN-229 cell migration and invasion. NUDT5 depletion decreased Ki-67, PCNA, *N*-cadherin, and vimentin expressions while increasing E-cadherin expression in comparison with the sh-NC group ($P<0.001$).

3.3. Interference with NUDT5 inhibits Warburg effect in GBM cells

ECAR assay showed that NUDT5 deficiency significantly reduced glycolysis from 30-70 min ($P<0.001$) (Figure 3A). Inhibition of NUDT5 resulted in a significant decrease in LDH level in LN-229 cells ($P<0.001$) (Figure 3B). Glucose consumption was also significantly reduced in the sh-NUDT5 group compared to the sh-NC group (Figure 3C). Moreover, ATP level was significantly increased ($P<0.01$) and the protein expressions of GLUT1 and HK2 were prominently downregulated ($P<0.001$) after silencing of NUDT5 (Figure 3D-F).

3.4. TRIM47 is highly expressed in GBM cells and interacts with NUDT5

GeneMANIA database found a potential interaction between NUDT5 and TRIM47 (Figure 4A). GEPIA database presented that TRIM47 expression was fortified in the tissues of GBM patients, and the elevated TRIM47 expression was notably correlated with the low overall survival rate of GBM patients (Figure 4B and C). In addition, a prominently positive correlation between NUDT5 and TRIM47 in GBM was displayed by GEPIA database (Figure 4D). RT-qPCR and Western blot analyses also demonstrated that TRIM47 mRNA and protein expressions were remarkably augmented in LN-229 cells ($P<0.001$) (Figure 4E and F). CO-IP results verified the targeted binding of NUDT5 to TRIM47 (Figure 4G and H). In addition, the

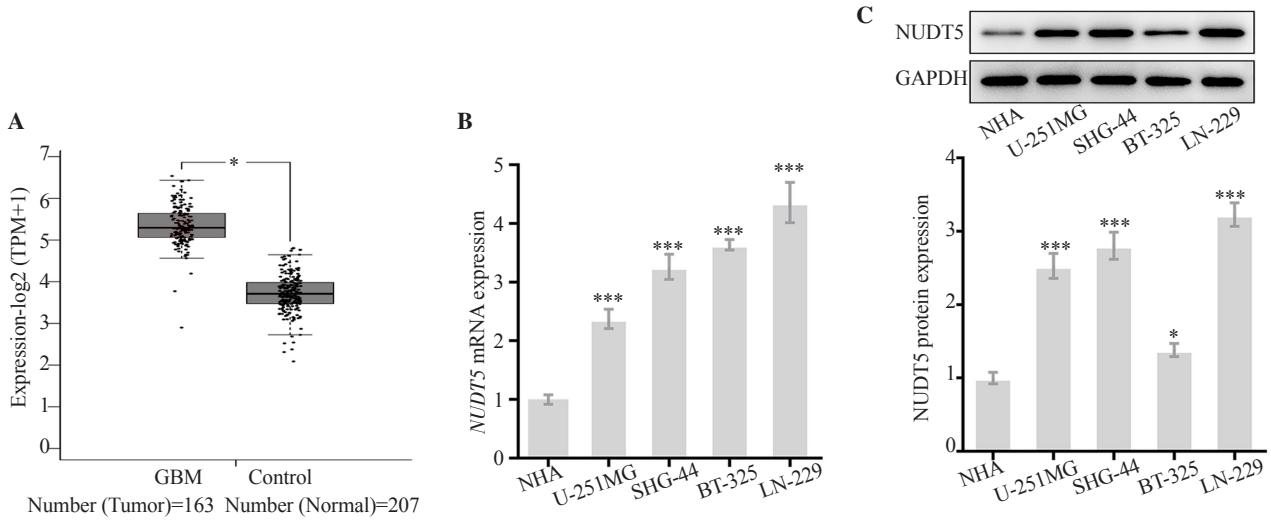

Figure 1. NUDT5 is overexpressed in multiple GBM. (A) The GEPIA database predicted the expression of NUDT5 in GBM. $^{*}P<0.05$ *vs*. the control. RT-qPCR (B) and Western blot (C) analyses of NUDT5 mRNA and protein expressions in GBM cells. $^{*}P<0.05$, $^{***}P<0.001$ *vs*. NHA. NUDT5: Nudix hydrolase 5; GBM: glioblastoma multiforme.

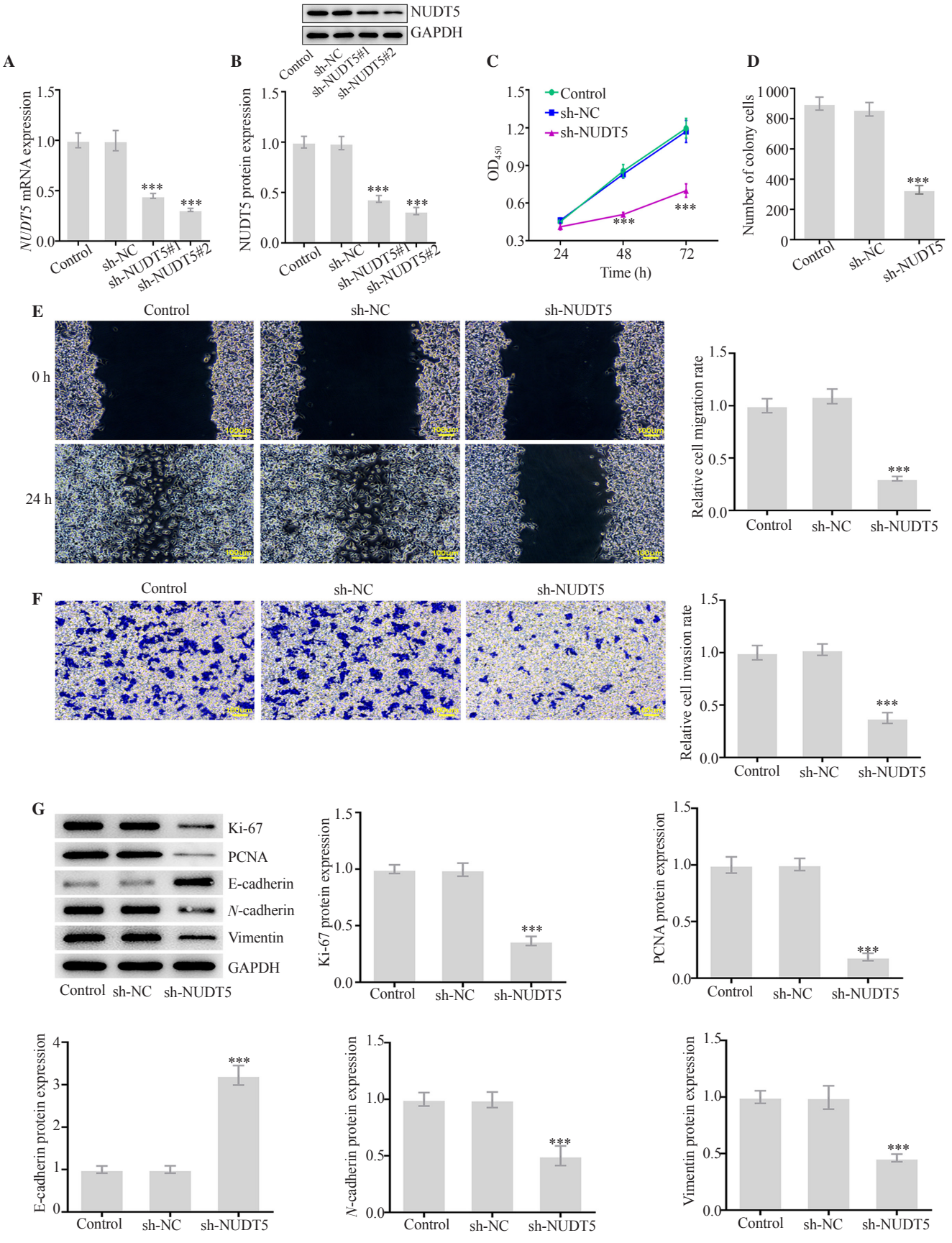

Figure 2. Interference with NUDT5 inhibits GBM cell proliferation, migration, and invasion. After transfection of short hairpin RNA targeting NUDT5 (sh-NUDT5) and the corresponding negative control (sh-NC), NUDT5 mRNA and protein expressions in GBM cells were determined by RT-qPCR (A) and Western blot (B) analyses. Cell viability and proliferation were evaluated by CCK-8 (C) and colony formation assay (D), respectively. Cell migration and invasion were evaluated by wound healing (E) and Transwell (F) assays, respectively. (G) Western blot analysis of proliferation-related proteins. $^{***}P<0.001$ *vs*. the sh-NC group.

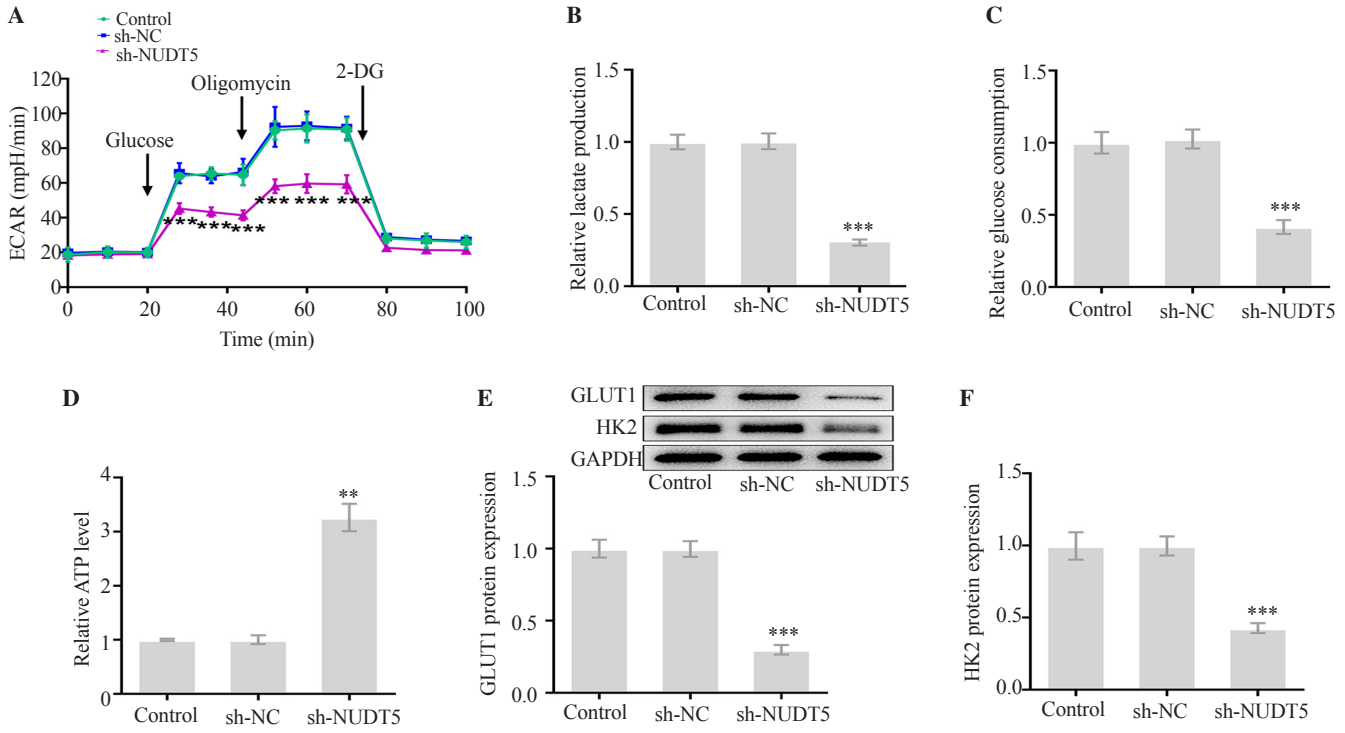

Figure 3. Interference with NUDT5 inhibits the Warburg effect in GBM cells. (A) The acid production rate was evaluated by ECAR assay. (B) An *L*-lactic acid kit was used to examine lactic acid level in the culture medium. (C) The level of glucose was measured by a glucose detection kit. (D) ATP level in cell supernatant was examined by ATP assay kit. (E-F) The expression of glycolysis-related proteins was determined by Western blot analysis. $^{**}P<0.01$, $^{***}P<0.001$ *vs*. the sh-NC group.

inhibition of NUDT5 lowered the expression of TRIM47 in LN-229 cells ($P<0.001$) (Figure 4I and J).

3.5. Overexpression of TRIM47 partially reverses the inhibitory effects of NUDT5 downregulation on GBM cell proliferation and metastasis as well as Warburg effect

In this experiment, TRIM47 expression was raised and RT-qPCR and Western blot tested the transfection efficiency (Figure 5A and B). To further study the effect of overexpressed TRIM47 on GBM cell proliferation and metastasis as well as Warburg effect after NUDT5 downregulation, four groups including the control, sh-NUDT5, sh-NUDT5 + Ov-NC and sh-NUDT5 + Ov-TRIM47 were used. CCK-8 and colony formation assay results showed that the cell viability in the sh-NUDT5 + Ov-TRIM47 group was significantly increased compared with that of the sh-NUDT5 + Ov-NC group ($P<0.001$) (Figure 5C and D). Wound healing and Transwell results showed that the migration and invasion of LN-229 cells in the sh-NUDT5 + Ov-TRIM47 group were significantly increased compared with the sh-NUDT5 + OV-NC group ($P<0.01$) (Figure 5E-F). Furthermore, compared with the sh-NUDT5 + Ov-NC group, TRIM47 elevation increased glycolysis (Figure 6A), lactate production (Figure 6B) and glucose consumption (Figure 6C), declined ATP level (Figure 6D), raised Ki-67, PCNA, *N*-cadherin, vimentin, GLUT1, and HK2 expressions and reduced E-cadherin (Figure 6E and F).

4. Discussion

In recent years, great advances have been achieved in systematic therapy (surgery, chemotherapy, and radiotherapy) for glioma, but the overall survival of GBM patients remains dissatisfactory[19]. It has been reported that various regulatory molecules participate in the occurrence and development of GBM. Nevertheless, the possible molecular mechanisms involving cellular behaviors including proliferation, metastasis, and glycolysis are still unclear[20]. Therefore, it is of great necessity to study the molecular mechanism of NUDT5 in regulating GBM proliferation, metastasis, and glycolysis.

In this paper, we found that the expression of NUDT5 in GBM was abnormally elevated through GEPIA database. We experimentally verified that NUDT5 expression was also abnormally elevated in GBM cell lines. High expression of NUDT5 has been demonstrated to predict unfavorable prognosis *via* mediating the AKT/cyclin D signaling pathway in breast cancer[21]. In patients with non-small-cell lung cancer, MTH1 and NUDT5 elevation contributed to tumor metastasis and suggested poor prognosis[22]. Therefore, it was speculated that NUDT5 might be implicated in the genesis and

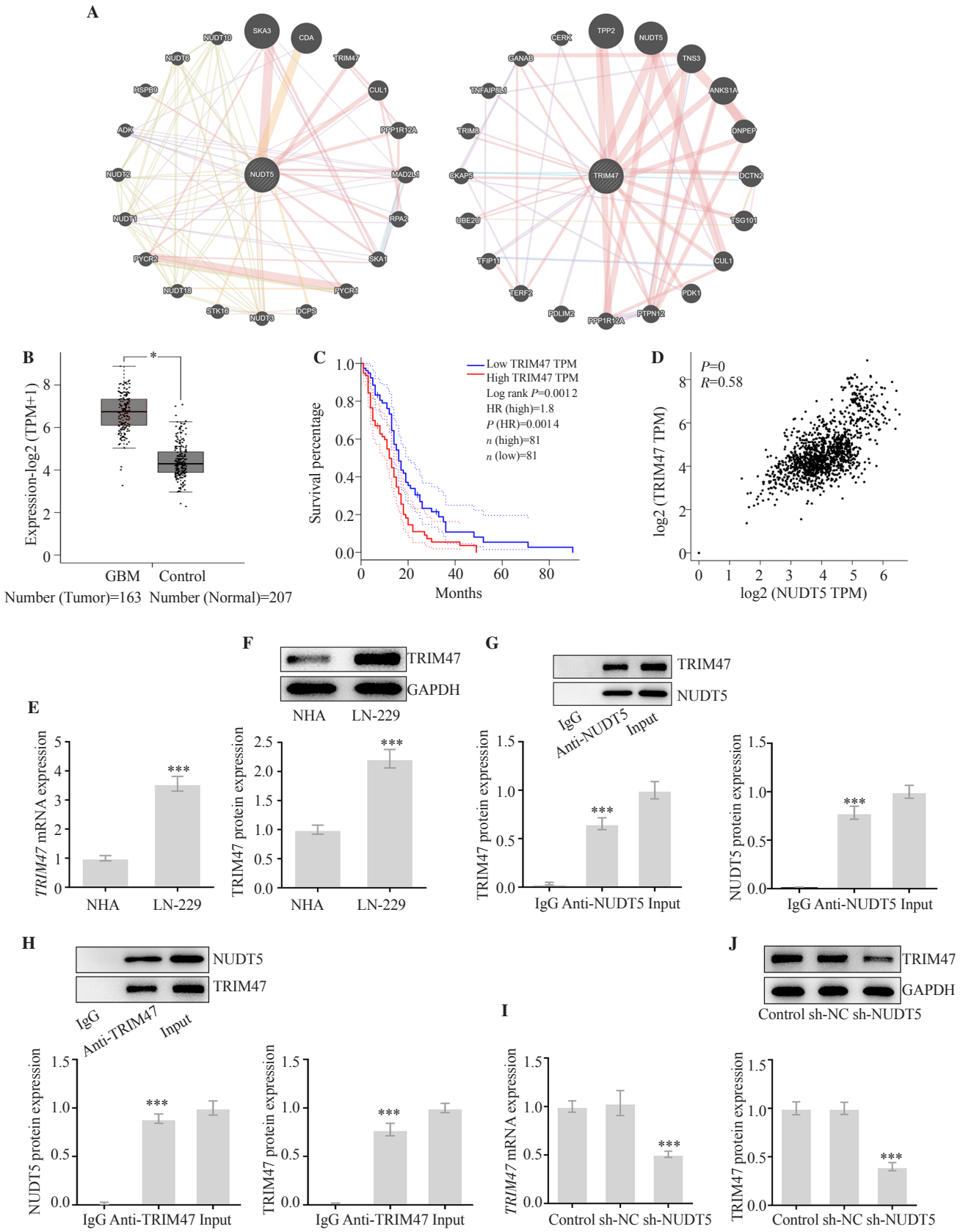

Figure 4. TRIM47 is highly expressed in GBM cells and interacts with NUDT5. (A) A potential interaction between NUDT5 and TRIM47 by GeneMANIA database. (B-D) The GEPIA database predicted the expression of TRIM47 in GBM and the correlation of its expression with the overall survival of GBM patients. $^{*}P<0.05$ *vs*. the control. NUDT5 was positively correlated with TRIM47 in GBM. TRIM47 mRNA and protein expressions in GBM cells were determined by RT-qPCR (E) and Western blot (F) analyses. $^{***}P<0.001$ *vs*. NHA. (G-H) The targeted binding of NUDT5 to TRIM47 was verified by co-immunoprecipitation. $^{***}P<0.001$ *vs*. the IgG group. TRIM47 mRNA and protein expressions in GBM cells were determined by RT-qPCR (I) and Western blot (J) analyses after silencing NUDT5. $^{***}P<0.001$ *vs*. the sh-NC group. TRIM47: tripartite motif family proteins 47.

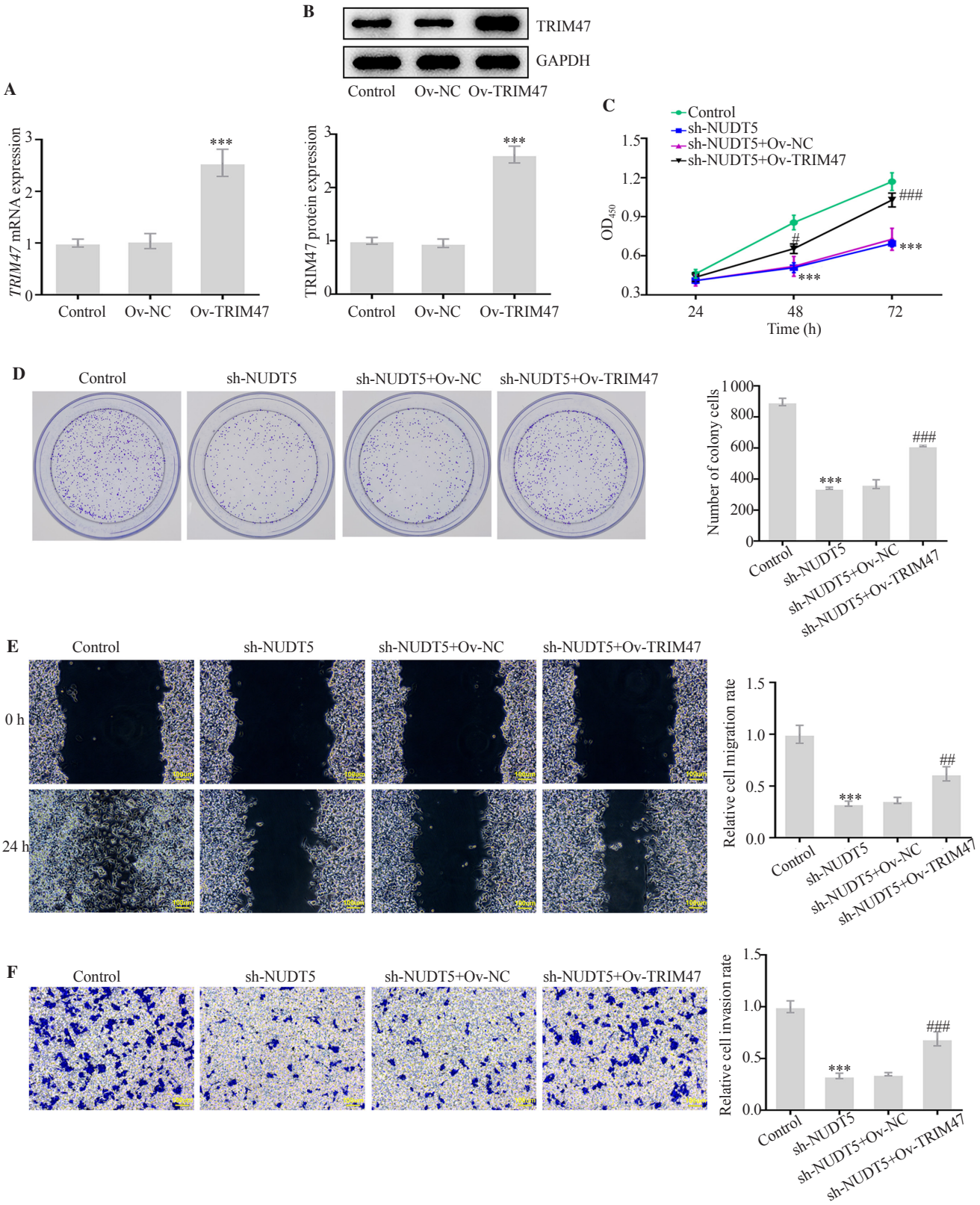

Figure 5. Overexpression of TRIM47 partially reverses the inhibitory effects of NUDT5 downregulation on GBM cell proliferation and metastasis. After transfection of overexpression plasmids specific to TRIM47 (Ov-TRIM47) and empty vector (Ov-NC) with a lentiviral expression vector GV 493, TRIM47 mRNA and protein expressions in GBM cells were determined by RT-qPCR (A) and Western blot (B) analyses. $^{***}P<0.001$ *vs*. the ov-NC group. CCK-8 (C) and colony formation assays (D) were performed to measure cell viability and proliferation. (E-F) Cell migration and invasion were evaluated by wound healing and Transwell assays, respectively. $^{***}P<0.001$ *vs*. the control, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs*. the sh-NUDT5 + ov-NC group.

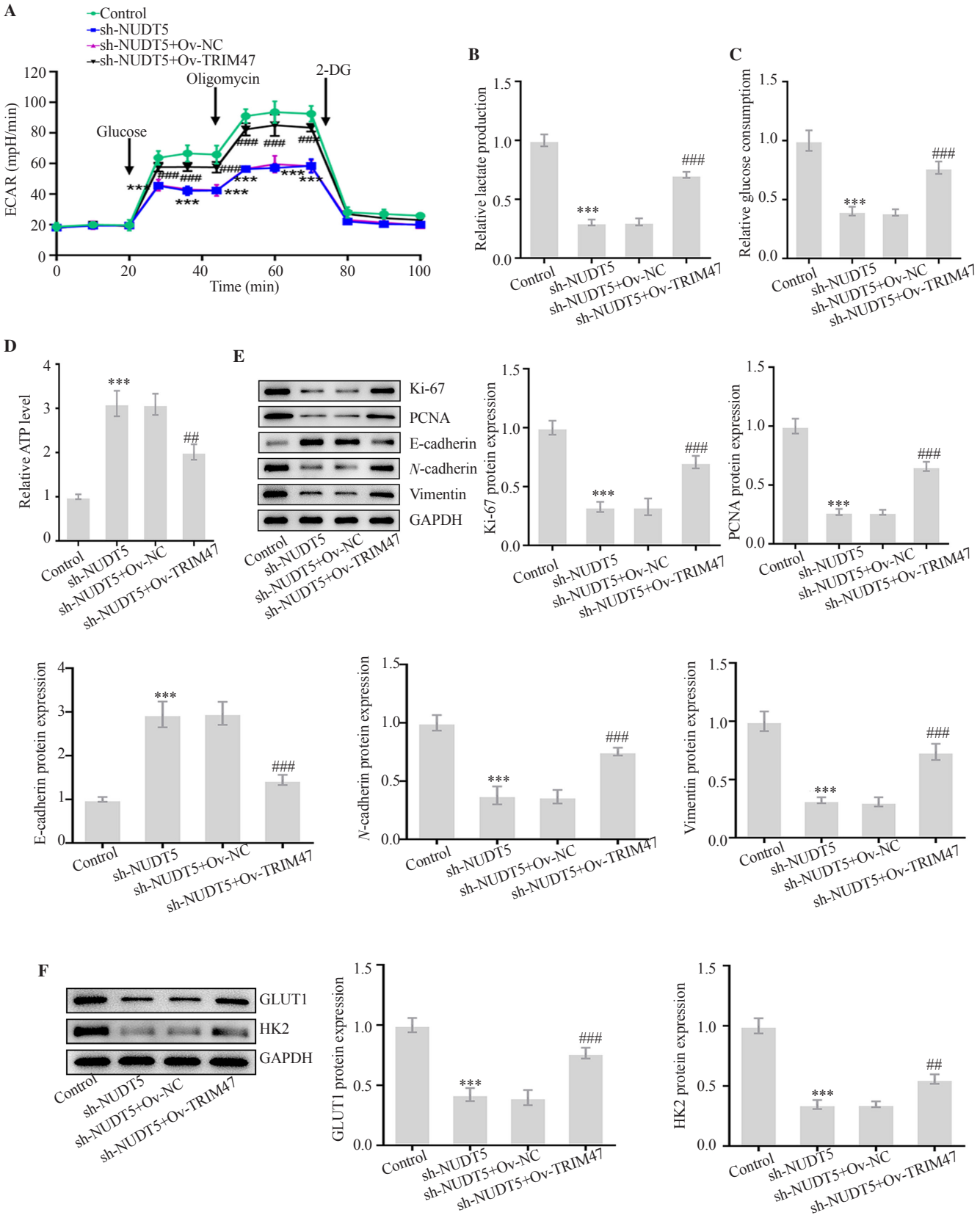

Figure 6. Overexpression of TRIM47 partially reverses the inhibition of NUDT5 downregulation on the Warburg effect in GBM cells. (A) The acid production rate was determined by ECAR assay. (B) The lactic acid level by *L*-lactic acid kit. (C) The level of glucose by glucose detection kit. (D) ATP level by ATP assay kit. The expressions of proliferation-related proteins (E) and glycolysis-related proteins (F) were determined by Western blot analysis. $^{***}P<0.001$ *vs*. the control group, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs*. the sh-NUDT5 + ov-NC group.

development of GBM. Therefore, NUDT5 expression was knocked down in LN-229 cells to explore the related mechanism. It was noticed that the absence of NUDT5 overtly suppressed GBM cell proliferation, invasion, and migration.

A common feature of tumor cells is a gradual increase in glucose intake and lactate accumulation, even under normal circumstances. Glycolysis, the primary energy metabolism way, has a higher sugar decomposition ability to convert glucose into lactic acid to produce ATP. We refer to the above phenomenon as the Warburg effect[3]. In our experiment, we found that NUDT5 inhibition could inhibit the Warburg effect in LN-229 cells[23].

GeneMANIA database showed a potential interaction between NUDT5 and TRIM47, which was then verified by CO-IP in this study. In addition, it was evidenced that NUDT5 had a positive correlation with TRIM47 in GBM. A previous study has shown that TRIM47 promoted the development of GBM through the ubiquitination and degradation of FOXO1[24]. TRIM47 depletion hindered the initiation and development of breast cancer through the inactivation of PI3K/Akt pathway[15]. TRIM47 was overexpressed in gastric cancer cells and was expected to be a new prognostic biomarker for gastric cancer patients[25]. In addition, TRIM47 mediated FBP1 ubiquitination to drive aerobic glycolysis and development of pancreatic cancer[14]. In our experiment, TRIM47 elevation was discovered to partially reverse the suppressive role of NUDT5 downregulation in GBM cell proliferation and metastasis as well as Warburg effect. However, our article has a limitation in that the effect of NUDT5 downregulation in animal experiments was not evaluated. Further study is needed to verify the role of NUDT5 in GBM.

In conclusion, our study found that NUDT5 facilitated the growth, metastasis, and Warburg effect of GBM by upregulating TRIM47. Both NUDT5 and TRIM47 can be used as targets for GMB treatment and our study provided a theoretical basis for exploring the pathogenesis of GBM and finding the targeted therapy of GBM in the clinic.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

ZFZ and SML conceived and designed the study. ZFZ carried out the experiments. SML analyzed the experimental data. ZFZ and SML wrote and revised the manuscript and confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

References

[1] Incekara F, Smits M, van der Voort SR, Dubbink HJ, Atmodimedjo PN, Kros JM, et al. The association between the extent of glioblastoma resection and survival in light of MGMT promoter methylation in 326 patients with newly diagnosed IDH-wildtype glioblastoma. *Front Oncol* 2020; **10**: 1087.

[2] Tirro E, Massimino M, Broggi G, Romano C, Minasi S, Gianno F, et al. A custom DNA-based NGS panel for the molecular characterization of patients with diffuse gliomas: Diagnostic and therapeutic applications. *Front Oncol* 2022; **12**: 861078.

[3] Caltabiano R, Torrisi A, Condorelli D, Albanese V, Lanzafame S. High levels of *connexin 43* mRNA in high grade astrocytomas. Study of 32 cases with *in situ* hybridization. *Acta Histochem* 2010; **112**(6): 529-535.

[4] Patil CG, Yi A, Elramsisy A, Hu J, Mukherjee D, Irvin DK, et al. Prognosis of patients with multifocal glioblastoma: A case-control study. *J Neurosurg* 2012; **117**(4): 705-711.

[5] Wang Y, Wan F, Chang K, Lu X, Dai B,Ye D. NUDT expression is predictive of prognosis in patients with clear cell renal cell carcinoma. *Oncol Lett* 2017; **14**(5): 6121-6128.

[6] Zhang L, Song XN, Dai DP, Zhou XY, Gan W, Takagi Y, et al. Lowered Nudix type 5 expression leads to cellular senescence in IMR-90 fibroblast cells. *Free Radic Res* 2013; **47**(6-7): 511-516.

[7] Zhang LQ, Dai DP, Gan W, Takagi Y, Hayakawa H, Sekiguchi M, et al. Lowered nudix type 5 (NUDT5) expression leads to cell cycle retardation in HeLa cells. *Mol Cell Biochem* 2012; **363**(1-2): 377-384.

[8] Wang JJ, Liu TH, Li J, Li DN, Tian XY, Ouyang QG, et al. The high expression of MTH1 and NUDT5 predict a poor survival and are associated with malignancy of esophageal squamous cell carcinoma. *Peer J* 2020; **8**: e9195. doi: 10.7717/peerj.9195.

[9] Tian Y, Chen L, Jiang Y. LASSO-based screening for potential prognostic biomarkers associated with glioblastoma. *Front Oncol* 2022; **12**: 1057383.

[10]Fujimura T, Inoue S, Urano T, Takayama K, Yamada Y, Ikeda K, et al. Increased expression of tripartite motif (TRIM) 47 is a negative prognostic predictor in human prostate cancer. *Clin Genitourin Cancer* 2016; **14**(4): 298-303.

[11]Li W, Zhang W, Deng W, Zhong Y, Zhang Y, Peng Z, et al. Quantitative proteomic analysis of mitochondrial proteins differentially expressed between small cell lung cancer cells and normal human bronchial

epithelial cells. *Thorac Cancer* 2018; **9**(11): 1366-1375.

[12]Liang Q, Tang C, Tang M, Zhang Q, Gao Y, Ge Z. TRIM47 is up-regulated in colorectal cancer, promoting ubiquitination and degradation of SMAD4. *J Exp Clin Cancer Res* 2019; **38**(1): 159.

[13]Chen L, Li M, Li Q, Xu M, Zhong W. Knockdown of TRIM47 inhibits glioma cell proliferation, migration and invasion through the inactivation of Wnt/beta-catenin pathway. *Mol Cell Probes* 2020; **53**: 101623.

[14]Li L, Yu Y, Zhang Z, Guo Y, Yin T, Wu H, et al. TRIM47 accelerates aerobic glycolysis and tumor progression through regulating ubiquitination of FBP1 in pancreatic cancer. *Pharmacol Res* 2021; **166**: 105429.

[15]Wang Y, Liu C, Xie Z, Lu H. Knockdown of TRIM47 inhibits breast cancer tumorigenesis and progression through the inactivation of PI3K/Akt pathway. *Chem Biol Interact* 2020; **317**: 108960.

[16]Li C, Tang Z, Zhang W, Ye Z, Liu F. GEPIA2021: Integrating multiple deconvolution-based analysis into GEPIA. *Nucleic Acids Res* 2021; **49**(W1): W242-W246.

[17]Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 2010; **38**(Web Server issue): W214-W220.

[18]Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001; **25**(4): 402-408.

[19]Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J Neuropathol Exp Neurol* 2005; **64**(6): 479-489.

[20]Wang Q, Lu X, Wang J, Yang Z, Hoffman RM, Wu X. Moesin up-regulation is associated with enhanced tumor progression imaged non-invasively in an orthotopic mouse model of human glioblastoma. *Anticancer Res* 2018; **38**(6): 3267-3272.

[21]Zhang H, Zhang LQ, Yang CC, Li J, Tian XY, Li DN, et al. The high expression of NUDT5 indicates poor prognosis of breast cancer by modulating AKT/Cyclin D signaling. *PLoS One* 2021; **16**(2): e0245876.

[22]Li DN, Yang CC, Li J, Ouyang QG, Zeng LT, Fan GQ, et al. The high expression of MTH1 and NUDT5 prom The high expression of MTH1 and NUDT5 promotes tumor metastasis and indicates a poor prognosis in patients with non-small-cell lung cancer. otes tumor metastasis and indicates a poor prognosis in patients with non-small-cell lung cancer. *Biochim Biophys Acta Mol Cell Res* 2021; **1868**(1): 118895.

[23]Liberti MV, Locasale JW. The Warburg effect: How does it benefit cancer cells? *Trends Biochem Sci* 2016; **41**(3): 211-218.

[24]Wei H, Ding C, Zhuang H, Hu W. TRIM47 promotes the development of glioma by ubiquitination and degradation of FOXO1. *Onco Targets Ther* 2020; **13**: 13401-13411.

[25]Xia Y, Wei Z, Huang W, Wei X, He Y. Trim47 overexpression correlates with poor prognosis in gastric cancer. *Neoplasma* 2021; **68**(2): 307-316.

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