YY1 transcription factor induces proliferation and aerobic glycolysis of neuroblastoma cells via LDHA regulation

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Abstract. The present study investigated the effect of transcription factor yin yang 1 (YY1) on aerobic glycolysis and cell proliferation in neuroblastoma and its mechanism. Neuroblastoma cell lines were used to investigate the association between YY1 and lactate dehydrogenase A (LDHA) expression. Cell Counting Kit (CCK)-8 and clone formation experiments were used to detect the cell viability. The interaction of YY1 and LDHA was detected using chromatin immunoprecipitation assay. Glucose uptake, intra/extracellular lactate and pyruvate and LDHA expression were evaluated using standard methods. Reverse transcription quantitative PCR, western blotting and gene overexpression or silencing were undertaken to explore the biological effects and underlying mechanisms of transcriptional regulators in NB cells. The results demonstrated that YY1 was significantly upregulated in neuroblastoma cell lines. The results of aerobic glycolysis and CCK-8 indicated that YY1 significantly promoted the proliferation and aerobic glycolysis of neuroblastoma cells. In addition, chromatin immunoprecipitation-PCR results demonstrated that YY1 was directly bound to the promoter LDHA. Overexpression of LDHA could reverse the inhibitory effect of sh-YY1 on aerobic glycolysis and proliferation of neuroblastoma cells. In conclusion, YY1 could induce aerobic glycolysis and proliferation of neuroblastoma cell lines, and may directly mediate the regulation of LDHA. These findings may provide novel insight for the treatment of neuroblastoma.

Introduction

Neuroblastoma is an extracranial solid tumor that often occurs in children and originates from the neural crest (1). Neuroblastoma accounts for ~15% of pediatric cancer-related mortalities worldwide (2). Aggressiveness and susceptibility to metastasis are key factors in neuroblastoma mortality (3). Multimodal treatment strategies such as high-dose marrow clearing chemotherapy and immunotherapy are currently used in clinical practice (4). However, patients who are cured still suffer from severe toxic side effects and drug resistance, severely affecting their quality of life (5). Therefore, it is clinically important to explore the molecular mechanisms of neuroblastoma and develop personalized treatment strategies for specific biomarkers.

Compared with normal cells, tumor cells have an altered metabolism, such as aerobic glycolysis (6). Under aerobic conditions, tumor cells take up glucose and convert it into large amounts of lactic acid, which improves the efficiency of ATP supply and promotes rapid cell proliferation (7). In addition, the energy required for tumor cell invasion and migration can be continuously supported by aerobic glycolysis. Reprogramming glucose metabolism from oxidative phosphorylation to aerobic glycolysis to promote cancer growth, a phenomenon known as the 'Warburg effect,' is a specific hallmark of tumor cell metabolism (8). Various enzymes and signaling molecules involved in aerobic glycolysis have been reported to have an important role in the malignant development of tumors (9). It has been reported (10) that microtubule affinity-regulating kinase 2 regulates mTOR/HIF-1α and p53 pathways to enhance aerobic glycolysis-mediated cell proliferation in breast cancer. Wu *et al* (11) reported that microRNA (miR)-489 targets downregulation of lactate dehydrogenase A (LDHA)-mediated aerobic glycolysis to inhibit multiple myeloma cell invasion and proliferation. Transcription factor yin yang 1 (YY1) can bind to gene promoters in a variety of cells, regulating downstream transcription and thereby affecting protein expression (12). Numerous reports (13-15) have demonstrated that YY1 is significantly upregulated in a variety of types of cancer, including breast cancer, lung cancer and glioma. YY1 can play a role in regulating cell proliferation, epithelial to mesenchymal transition and chemoresistance. However, to the best of our knowledge, the mechanism of action of YY1 in regulating aerobic glycolysis in neuroblastoma has been rarely reported.

The present study investigated YY1-mediated LDHA regulation of cell aerobic glycolysis and cell proliferation in neuroblastoma cells. The potential regulatory mechanism of YY1 may provide a novel therapeutic strategy for the development of new treatments for neuroblastoma.

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Materials and methods

Cell culture and cell transfection. Normal dorsal root ganglia (DG), neuroblastoma cell lines (BE(2)-C, IMR-32, SH-SY5Y and SK-N-AS) were purchased from the American Type Culture Collection. Cells were cultured in RPMI-1640 with 10% FBS and 100 μg/ml each of penicillin and streptomycin (all from Procell Life Science & Technology Co., Ltd.) at 37˚C in 70% humidity consisting of 5% CO_2.

Short hairpin (sh)-YY1 (sense, 5'-CCAAACAACUGG CAGAAUUTT-3'; anti-sense, 5'-AAUUCUGCCAGUUGU UUGGTT-3'), Sh negative control (sh-NC; sense, 5'-UUC UCCGAACGUGUCACGUTT-3'; antisense, 5'-ACGUGA CACGUUCGGAGAATT-3'), pcDNA3.1 and pcDNA3.1-YY1 (50 nM) were synthesized by Shanghai GenePharma Co., Ltd. When the neuroblastoma cell confluence reached 70%, lipofectamine 3000 (Thermo Fisher Scientific, Inc.) was used at 37˚C for 24 h according to the instructions. At 48 h post-transfection, subsequent experiments were performed.

293 T cells were transfected with either pGL3.0 basic or pcDNA3.1-YY1 plasmids (Shanghai GeneChem Co., Ltd.) together with the reporter plasmids pGL3.0-Basic-LDHA-promoter (-2000, -1500, -1200, -1000, -500 and -1200 mut). The second generation system was used to package the virus. In brief, 293 T cells were infected with 1 μg purified lentiviral particles expressing YY1 (LV-YY1-puromycin; Shanghai GeneChem Co., Ltd) or negative control (NC) lentiviral particles (LV-puromycin; Shanghai GeneChem Co., Ltd) at an MOI of 20 along with pMD2G (envelope plasmid) and ps-PAX2 (packaging plasmid) (4:1:3). After 48 h transfection, 293 T cells were treated with 1 μg/ml puromycin at 37˚C for 24 h and were harvested for RT-qPCR validation and the follow-up experiment.

Cell Counting Kit-8 (CCK-8) assay. The cell viability of neuroblastoma cells was detected using CCK-8 kit (Beyotime Institute of Biotechnology) after 24 h culture. In simple terms, cells were tested in a 96-well plate with 10^4 cells/well and 70% fusion of cultured cells. Subsequently, 10 μl CCK-8 reagent was added to each well, and incubate for 3 h in the incubator at 37˚C. Finally, the absorbance at 450 nm was measured using a microplate reader.

Colony formation experiment. The transfected cells (SH-SY5Y and IMR-32) were inoculated into six-well plates with 400 cells per well and cultured at 37˚C in complete medium (Procell Life Science & Technology Co., Ltd.) for 2 weeks. When colonies with >50 cells were visible, they were fixed with 4% paraformaldehyde at room temperature for 15 min and stained with crystal violet for 10 min at room temperature. Finally, colonies were observed under a microscope and images were captured. A total of three random fields were selected for counting and analysis using Image J software (v1.52s; National Institutes of Health).

Chromatin immunoprecipitation assay (ChIP). The binding of YY1 to the promoter of LDHA was verified using ChIP analysis. ChIP was performed according to the methods described previously (16).

Bioinformatics analysis. The Human Transcription Factor Database (http://Bioinfo.life.hust.edu.cn/HumanTFDB/#!) was used to predict the binding site of YY1 on the promotor region of LDHA.

Western blotting assay. The proteins were extracted from the neuroblastoma cells using RIPA lysis buffer (Thermo Fisher Scientific, Inc.). According to the kit instructions, the protein sample concentration was determined using BCA kit (Thermo Fisher Scientific, Inc.) and diluted to the same concentration using PBS. A total of 30 μg protein samples per lane were separated using 10% SDS-PAGE electrophoresis and transferred to polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was sealed with 5% skim milk for 3 h at room temperature and then incubated with the following primary antibodies: Anti-YY1 (cat. no. ab109237) anti-GLUT1 (cat. no. ab196357), anti-HK2 (cat. no. ab209847), anti-PDHK1 (cat. no. ab202468) and anti-LDHA (cat. no. ab125683) (all 1:1,000; Abcam) at 4˚C overnight. The next day, the membrane was washed with TBST (1% Tween-20) and incubated with the secondary anti-rabbit IgG antibody (1:10,000) (cat. no. ZF-0314; OriGene Technologies, Inc.) at 37˚C for 2 h. Finally, the protein expression intensity was detected using chemiluminescence reagent (Thermo Fisher Scientific, Inc.) in the dark, and the gray value was measured using ImageJ software (v1.52s, National Institutes of Health) to evaluate the relative protein expression.

Reverse transcription quantitative PCR (RT-qPCR). Total RNA was extracted from transfected cell (SH-SY5Y and IMR-32) samples using TRIzol® reagents (Invitrogen; Thermo Fisher Scientific, Inc.), and PrimeScriptTM cDNA kit (Takara Biotechnology Co., Ltd.) was used to create cDNA in accordance with the manufacturer's instructions. Utilizing an ABI 7000 Prism Step One plus detection system, RT-qPCR results were obtained. The standard qPCR procedure, as follows: Denaturation at 95˚C for 5 min; followed by 38 cycles of 95˚C for 30 sec, 58˚C for 30 sec and 72˚C for 20 sec; and a final step of 72˚C for 7 min. The primer sequences used were the following: YY1 forward, 5'-AGCAGAAGCAGGTGCAGA TCAA-3', and reverse, 5'-CTGCCAGTTGTTTGGGATCT-3'; GLUT1 forward, 5'-CTATGGGGAGAGCATCCTGC-3', and reverse, 5'-CCCAGTTTCGAGAAGCCCAT-3'; LDHA forward, 5'-CGTCGATATTCCTTTTCCACG-3', and reverse, 5'-AGCAAGTTCATCTGCCAAGTC-3'; HK-2 forward, 5'-AAGGCTTCAAGGCATCTG-3', and reverse, 5'-CCACAG GTCATCATAGTTCC-3'; PDHK2 forward, 5'-GCAAGTTCT CCCCGTCCCCG-3', and reverse, 5'-GGACATACCAGCTCT GCACCAG-3'; GAPDH forward, 5'-TGGACTCCACGACGT ACTCAG-3', and reverse, 5'-ACATGTTCCAATATGATT CCA-3'; GAPDH was used as an internal reference gene, the relative expression was measured using the $2^{-\Delta\Delta Cq}$ method (17).

Aerobic glycolysis assays. Cellular glucose uptake and lactate production were detected as previously described (18). After transfected cells were cultured for 48 h, cell culture supernatants were collected and assayed for glucose uptake and lactate accumulation according to the instructions of the glucose uptake assay kit (cat. no. ab136955; Abcam) and lactate kit (cat. no. ab287808; Abcam). Relative glucose uptake and

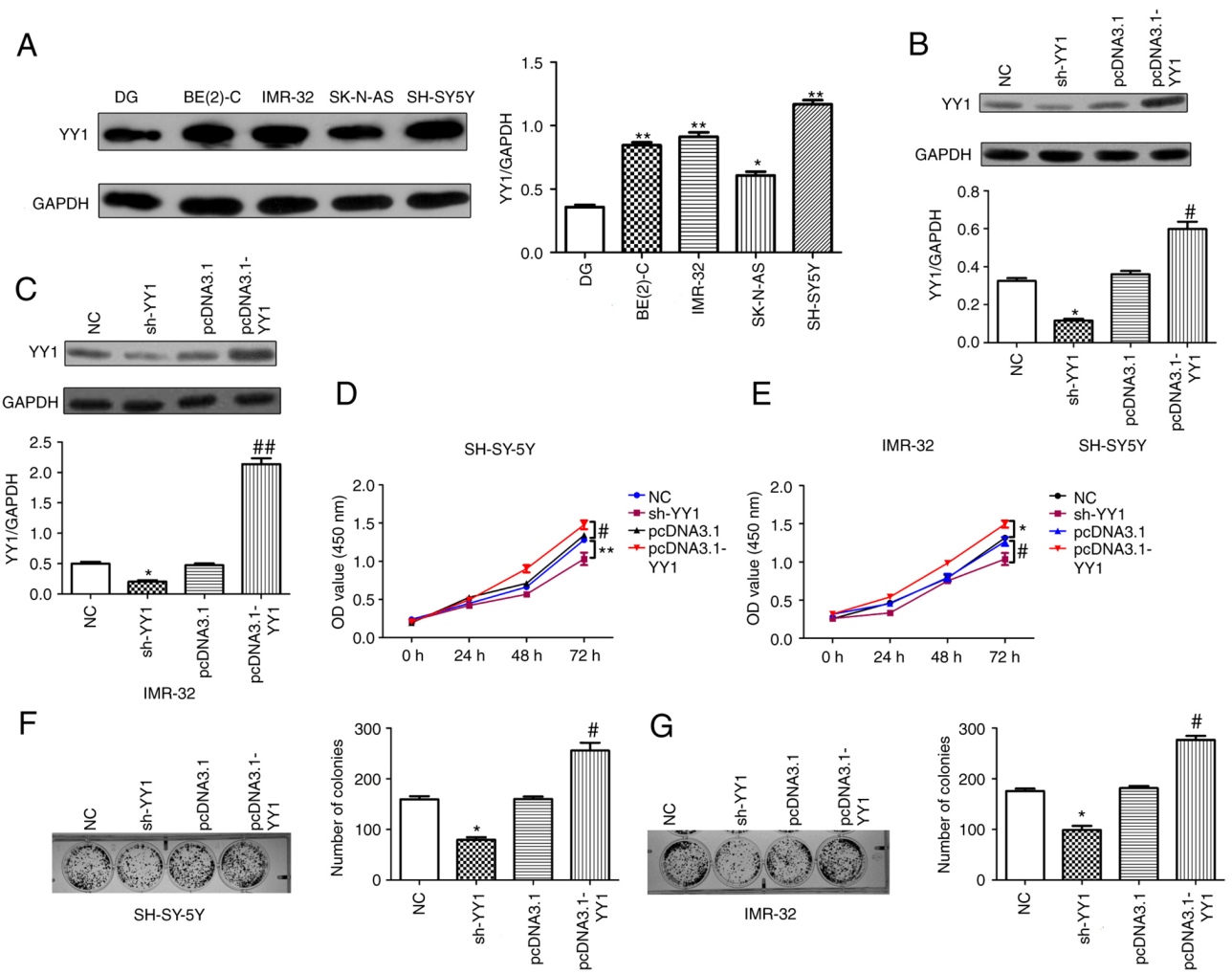

Figure 1. YY1 promotes neuroblastoma cells proliferation. (A) Western blotting assay was used to detect the expression levels of YY1 in neuroblastoma cell lines. Western blotting was used to detect the expression of YY1 in (B) SH-SY-5Y and (C) IMR-32 cells. Cell Counting Kit-8 as used to detect the cell viability in (D) SH-SY-5Y and (E) IMR-32. Colony formation assay was used to detect cell proliferation in (F) SH-SY-5Y and (G) IMR-32. *P<0.05 and **P<0.01 vs. DG/NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. pcDNA3.1 group. YY1, yin yang 1; DG, dorsal root ganglia; NC, negative control; OD, optical density.

lactate production were assessed according to the standard curve based on the determination of a standard sample.

Statistical analysis. The data in the present study was presented as mean ± standard deviation. The SPSS 22.0 software (IBM Corp.) package was used for statistical analysis. A one-way ANOVA followed by Tukey's post-hoc test was performed for comparisons between ≥3 groups. P<0.05 was considered to indicate a statistically significant difference.

Results

YY1 promotes proliferation of neuroblastoma cells. First, RT-qPCR analysis was used to detect the mRNA expression levels of YY1 in different cells. As presented in Fig. 1A, it confirmed that the mRNA expression levels of YY1 in SH-SY5Y cells and IMR-32 were significantly higher compared with DG cells. Therefore, in subsequent experiments, SH-SY5Y cells and IMR-32 cells were selected for the experiment. Western blotting results demonstrated that compared with NC group the expression of YY1 was significantly lower in sh-YY1 group in both SH-SY5Y cells and IMR-32 cell lines. In addition, the expression of YY1 in pcDNA3.1-YY1 group was significantly higher compared with pcDNA3.1 group (Fig. 1B and C). The aforementioned results indicated that knockdown and overexpression of YY1 were successfully transfected. Subsequently, CCK-8 kit was used to detect the cell viability. Compared with NC group, the cell viability in sh-YY1 group was significantly inhibited. While, the cell viability in pcDNA3.1-YY1 group was significantly increased compared with that in pcDNA3.1 group (Fig. 1D and E). Colony formation assay was used to detect cell proliferation in SH-SY5Y cells and IMR-32. The results (Fig. 1F and G) demonstrated that compared with NC group, the ability of colony formation in sh-YY1 group was significantly reduced. While, compared with pcDNA3.1 group, the ability of clone formation in pcDNA3.1-YY1 group was significantly increased. Overall, YY1 could significantly promote cell proliferation in SH-SY5Y and IMR-32 cells.

YY1 regulates aerobic glycolysis in neuroblastoma cells. The lactate production and relative glucose uptake was measured to demonstrate that YY1 regulated aerobic glycolysis of neuroblastoma cells. As presented in Fig. 2A and B, compared

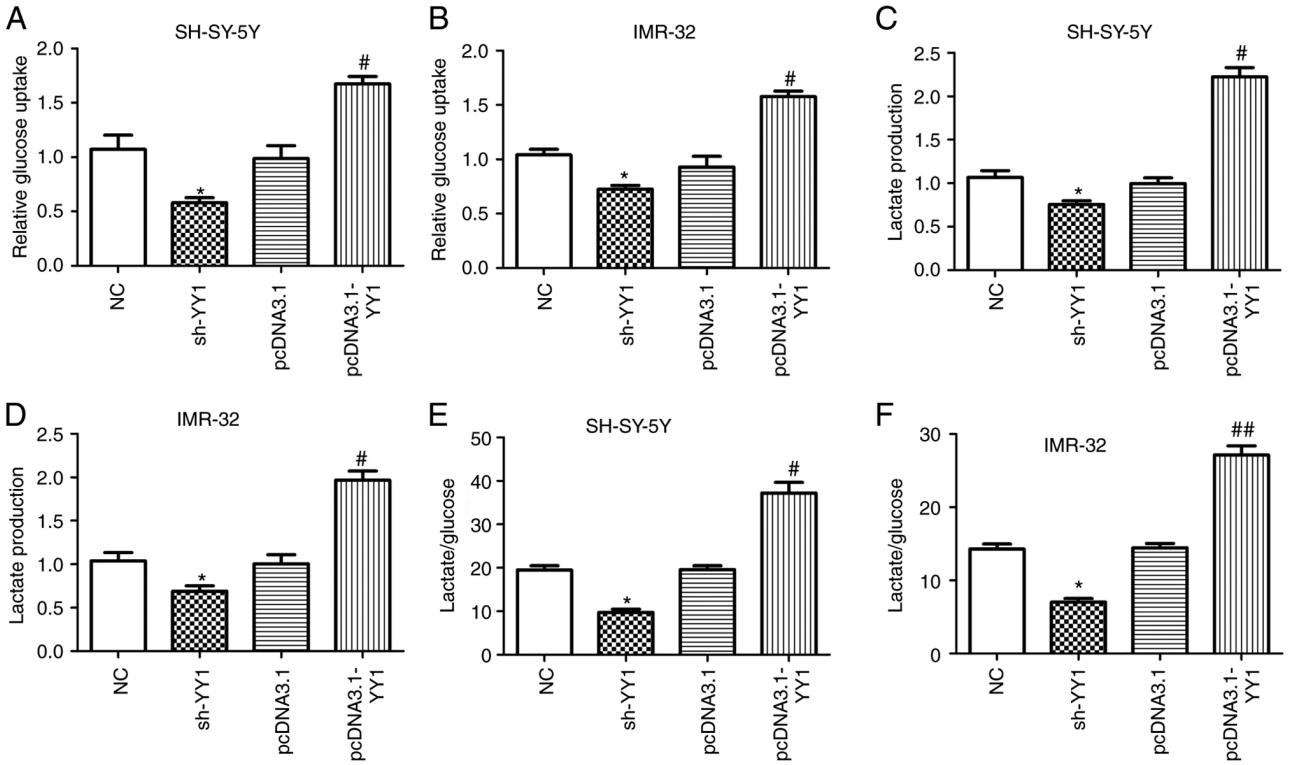

Figure 2. YY1 regulates aerobic glycolysis in neuroblastoma cells. Relative glucose uptake and lactate production in SH-SY-5Y and IMR-32 cells were detected. Glucose uptake for (A) SH-SY-5Y and (B) IMR-32. Lactate production for (C) SH-SY-5Y and (D) IMR-32. The ratio of glucose uptake to lactate production for (E) SH-SY-5Y and (F) IMR-32. *P<0.05 vs. NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. pcDNA3.1 group. YY1, yin yang 1; NC, negative control; sh, short hairpin.

with NC group, the relative glucose uptake was significantly reduced in sh-YY1 group in both cell lines. Meanwhile, the relative glucose uptake in pcDNA3.1-YY1 group was significantly increased compared with pcDNA3.1 group. Similarly, Fig. 2C and D demonstrated that compared with NC group, lactate production was significantly reduced in sh-YY1 group. In addition, the lactate production in pcDNA3.1-YY1 group was increased significantly compared with pcDNA3.1 group. Lactate-glucose ratio was measured in different groups (Fig. 2E and F). Compared with NC group, the lactate-glucose ratio was reduced significantly in sh-YY1 group. While, the lactate-glucose ratio in pcDNA3.1-YY1 group was increased significantly compared with pcDNA3.1 group. The results indicated that YY1 significantly increased the lactate-glucose ratio in neuroblastoma cells (SH-SY5Y and IMR-32). Overall, this suggested that YY1 regulated aerobic glycolysis in neuroblastoma cells.

YY1 regulates glycolytic gene expression in neuroblastoma cells. In order to further study the effect of YY1 on aerobic glycolysis, the expression levels of associated genes were detected using RT-qPCR analysis. The results indicated that the mRNA expressions of glucose transporter 1 (GLUT1), hexokinase 2 (HK-2), LDHA and pyruvate dehydrogenase (PDHK1) in sh-YY1 group were reduced significantly compared with NC group (Fig. 3A and B). While, compared with pcDNA3.1 group, the mRNA expressions of GLUT1, HK-2, LDHA and PDHK1 in pcDNA3.1-YY1 group were significantly increased. Western blotting results indicated the same trend as RT-qPCR results (Fig. 3C and D). Overall, this revealed that YY1 could regulate glycolytic gene expression in neuroblastoma cells.

Transcription factor YY1 binds to LDHA's promoter. According to the bioinformatics analysis, the interaction of YY1 and LDHA was detected using CHIP assay. As presented in Fig. 4A, it indicated that there is a potential binding site at -2000 bp to -500 bp. The CHIP-PCR assay was performed using anti-YY1 anti-body. CHIP results revealed ~8-fold enrichment of the YY1-bound LDHA promoter compared with IgG group (Fig. 4B). The results indicated that transcription factor YY1 binds to LDHA's promoter.

Overexpression of LDHA can alleviate the inhibitory effect of sh-YY1 on the proliferation of neurocytoma cells. Western blotting was performed to detect the protein expression levels of LDHA in neuroblastoma cells. As presented in Fig. 5A and B, the expression of LDHA in sh-YY1 + pcDNA3.1 group was reduced significantly compared with NC group in both SH-SY5Y and IMR-32 cell lines. Moreover, compared with sh-YY1 + pcDNA3.1 group, the expression of LDHA in sh-YY1+ pcDNA3.1-LDHA group was significantly increased. Furthermore, CCK-8 assay was used to detect the cell viability in different groups. The results demonstrated that the cell viability in sh-YY1 + pcDNA3.1 group was significantly inhibited compared with NC group (Fig. 5C and D). The cell viability in sh-YY1 + pcDNA3.1-LDHA group was increased significantly compared with sh-YY1 + pcDNA3.1 group. Colony formation results demonstrated that, relative to NC group, the

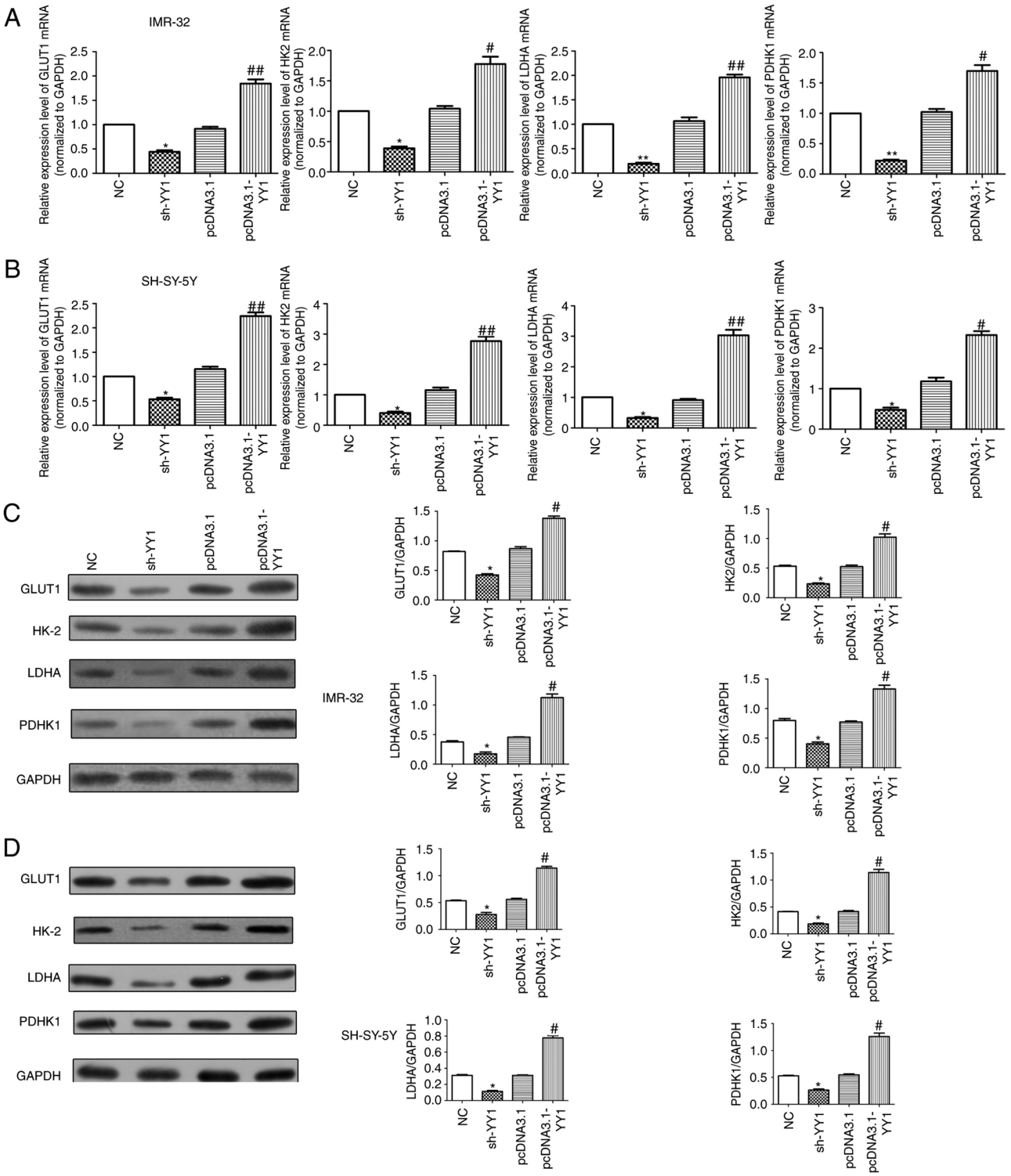

Figure 3. YY1 regulates glycolytic gene expression in neuroblastoma cells. Reverse transcription-quantitative PCR analysis was performed to detect the mRNA expression levels of GLUT1, HK-2, LDHA and PDHK1 in (A) IMR-32 and (B) SH-SY-5Y cells. Western blotting was used to detect the expression levels of GLUT1, HK-2, LDHA and PDHK1 in (C) IMR-32 and (D) SH-SY-5Y cells. *P<0.05 vs. NC group; **P<0.01; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. pcDNA3.1 group. YY1, yin yang 1; NC, negative control; sh, short hairpin; GLUT1, glucose transporter 1; HK-2, hexokinase 2; LDHA, lactate dehydrogenase A; PDHK1, pyruvate dehydrogenase.

proliferation in sh-YY1 + pcDNA3.1 group was significantly inhibited (Fig. 5E and F). The proliferation in sh-YY1 + pcDNA3.1-LDHA group was increased significantly compared with sh-YY1 + pcDNA3.1 group. According to the above results, YY1 was considered to block the decline of proliferation by LDHA knockdown in neuroblastoma cells.

Overexpression of LDHA can reverse the inhibitory effect of sh-YY1 on aerobic glycolysis in neuroblastoma cells. After transfection, the lactate production and relative glucose uptake were measured to demonstrate that YY1 regulated aerobic glycolysis of neuroblastoma cells. As presented in Fig. 6A, the relative glucose uptake was reduced significantly in sh-YY1 +

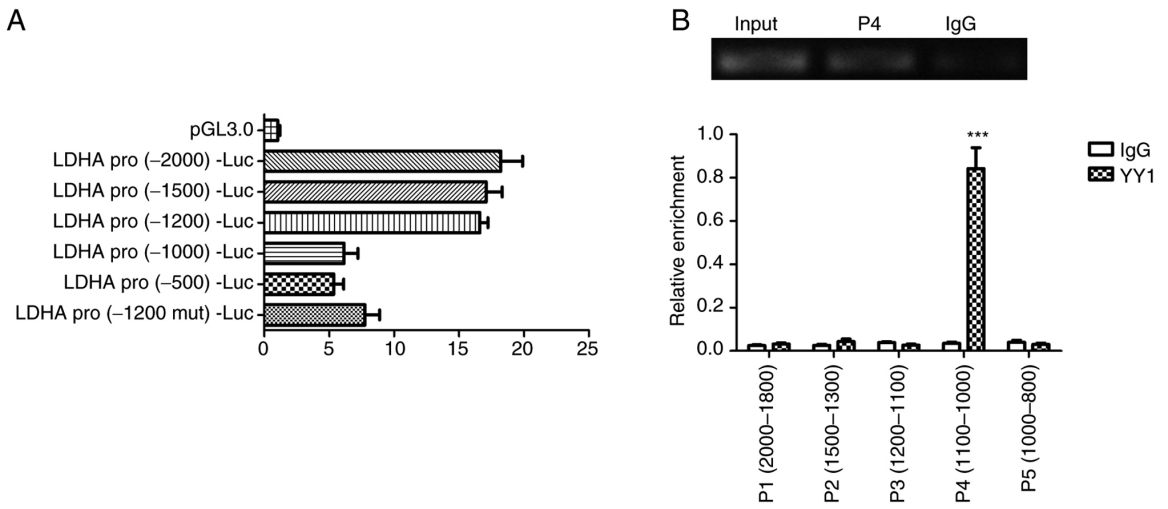

Figure 4. Transcription factor YY1 binds to LDHA's promoter. (A) 293 T cells were transfected with either pGL3.0 basic or pcDNA3.1-YY1 plasmids together with the reporter plasmids pGl3.0-Basic-LDHA-promoter (-2000, -1500, -1200, -1000, -500 and -1200 mut). (B) Chromatin immunoprecipitation-PCR assay was used to detect the relative enrichment. ***P<0.001 vs. IgG group. LDHA, lactate dehydrogenase A; YY1, yin yang 1.

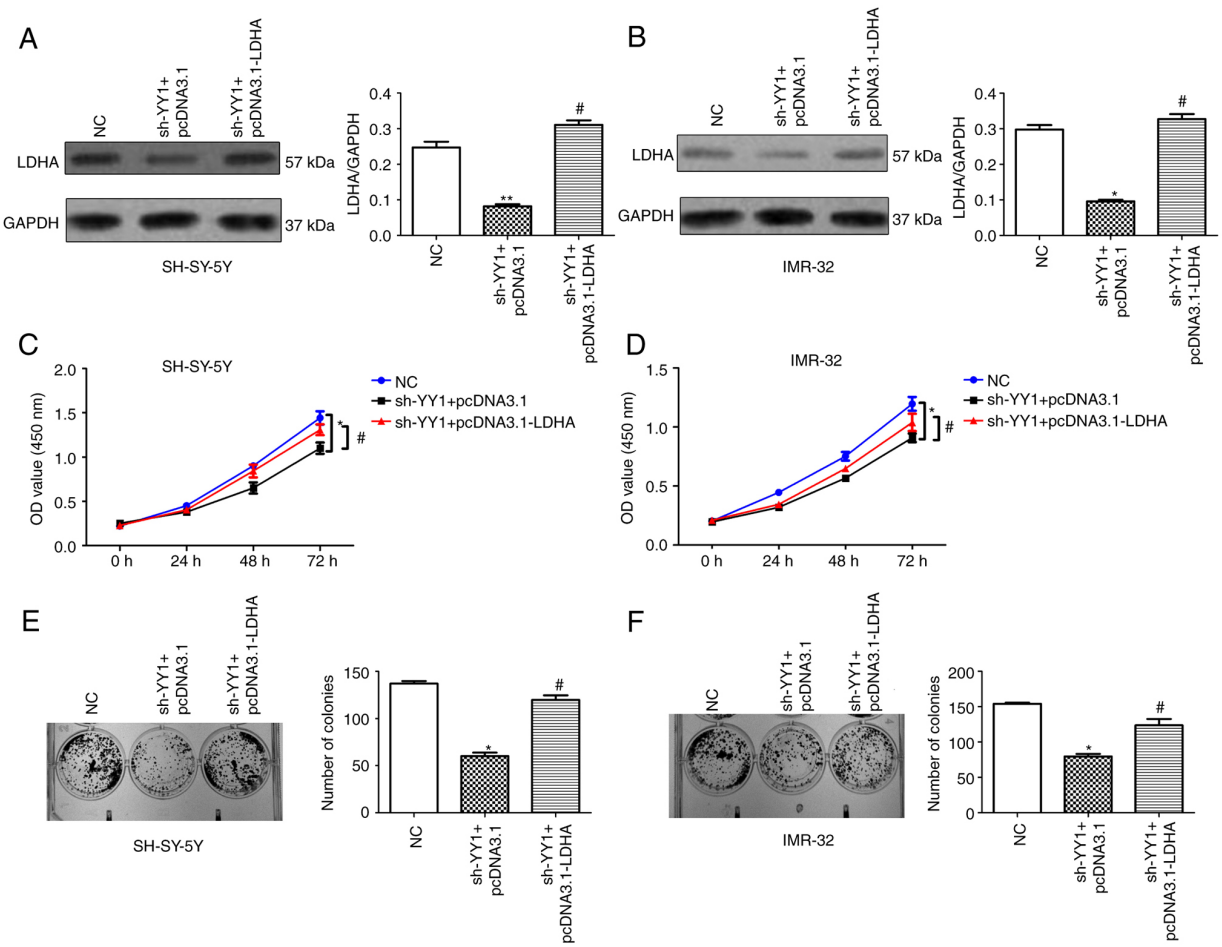

Figure 5. Overexpression of LDHA can alleviate the inhibitory effect of sh-YY1 on the proliferation of neurocytoma cells. Western blotting assay was used to detect the expression of YY1 in (A) SH-SY-5Y and (B) IMR-32 cells. Cell Counting Kit-8 kit was used to detect the cell viability in (C) SH-SY-5Y and (D) IMR-32. Colony formation assay was used to detect cell proliferation in (E) SH-SY-5Y and (F) IMR-32. *P<0.05 and **P<0.01 vs. NC group; #P<0.05 vs. pcDNA3.1 group. LDHA, lactate dehydrogenase A; YY1, yin yang 1; NC, negative control; OD, optical density; sh, short hairpin.

pcDNA3.1 group compared with NC group in both SH-SY5Y and IMR-32 cell lines. While the relative glucose uptake in sh-YY1 + pcDNA3.1-LDHA group was significantly increased compared with pcDNA3.1 group. Similarly, Fig. 6B demonstrated that lactate production was reduced significantly in sh-YY1 + pcDNA3.1 group compared with NC group. The

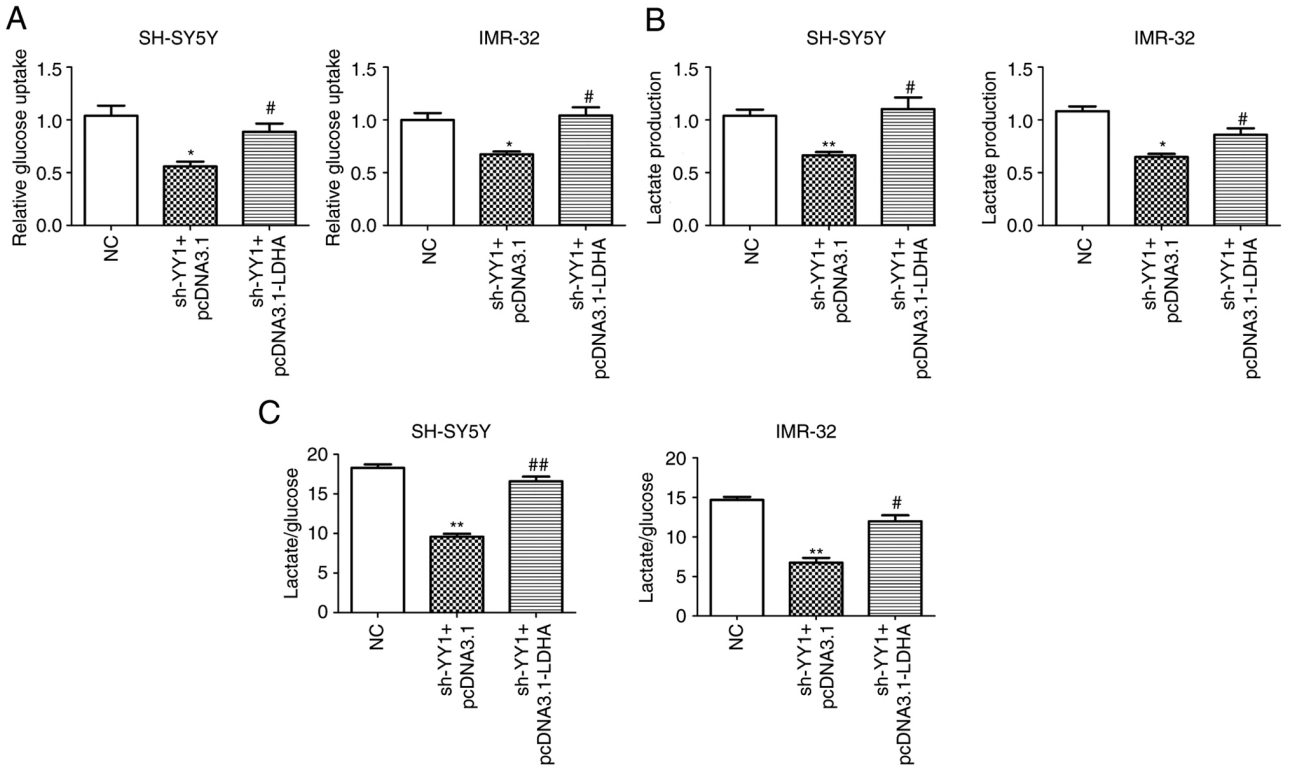

Figure 6. Overexpression of LDHA can reverse the inhibitory effect of sh-YY1 on aerobic glycolysis in neuroblastoma cells. Relative glucose uptake and lactate production in SH-SY-5Y cells were detected. (A) Glucose uptake for SH-SY-5Y and IMR-32. (B) Lactate production for SH-SY-5Y and IMR-32. (C) Ratio of glucose uptake to lactate production for SH-SY-5Y and IMR-32. *P<0.05 and **P<0.01 vs. NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. pcDNA3.1 group. LDHA, lactate dehydrogenase A; YY1, yin yang 1; NC, negative control; sh, short hairpin.

lactate production in sh-YY1 + pcDNA3.1-LDHA group was significantly increased compared with sh-YY1 + pcDNA3.1 group. In addition, lactate-glucose ratio was measured in different groups (Fig. 6C). Compared with NC group, the lactate-glucose ratio in sh-YY1 + pcDNA3.1 group was reduced significantly. While the lactate-glucose ratio in sh-YY1 + pcDNA3.1-LDHA group was increased significantly compared with sh-YY1 + pcDNA3.1 group. The results indicated that YY1 blocked the decline of aerobic glycolysis by LDHA knockdown in neuroblastoma cells.

Overexpression of LDHA can reverse the regulatory effect of sh-YY1 on the expression of aerobic glycolytic-associated molecules in neuroblastoma cells. RT-qPCR results (Fig. 7A and B) indicated that the mRNA expression levels of GLUT1, HK-2, LDHA and PDHK1 in sh-YY1 + pcDNA3.1-LDHA group were significantly increased compared with sh-YY1 + pcDNA3.1 group. Western blotting results demonstrated the same trend, which was consistent with the RT-qPCR results (Fig. 7C and D). In summary, this confirmed that LDHA could reverse the regulatory of sh-YY1 on the expression of aerobic glycolytic-associated molecules in neuroblastoma cells.

Discussion

Neuroblastoma is a serious threat to the health of the child due to its high recurrence and poor prognosis (19). Aerobic glycolysis has been reported (20) to play an important role in the proliferation, invasion and metastasis of cancer cells. However, the molecular mechanism of transcriptional regulation of aerobic glycolysis in neuroblastoma is still unclear. The present study investigated the molecular mechanism of transcription factor YY1 in regulating aerobic glycolysis in neuroblastoma. It has been reported that the inhibition of YY1 can inhibit the proliferation of neuroblastoma cells (21). The present results indicated that YY1 was significantly upregulated in neuroblastoma cells and that overexpression of YY1 promoted neuroblastoma cell proliferation. In addition, ChIP experiments confirmed that the transcription factor YY1 could target binding to the LDHA promoter. These findings suggested that YY1 regulated aerobic glycolysis and promoted neuroblastoma cell proliferation through LDHA.

YY1 is a ubiquitous transcription factor that plays an important role in development. YY1 is associated with cell proliferation, anti-apoptosis, tumor invasion and metastasis (22-24). YY1 directly binds to and activates PFKP, a gene encoding a glycolytic rate-limiting enzyme that significantly promotes the Warburg effect and malignant growth forced by YY1 in advanced prostate cancer (14). YY1 promotes the Warburg effect in tumor development by regulating the glucose transporter GLUT3 (25). The present study revealed that inhibition of YY1 significantly inhibited the proliferation in neuroblastoma.

Aerobic glycolysis, a unique metabolic mode of cancer cells, can accelerate cancer cell proliferation (26). Targeted inhibition of aerobic glycolysis-related signaling molecules such as GLUT1, HK-2 and LDHA have been indicated

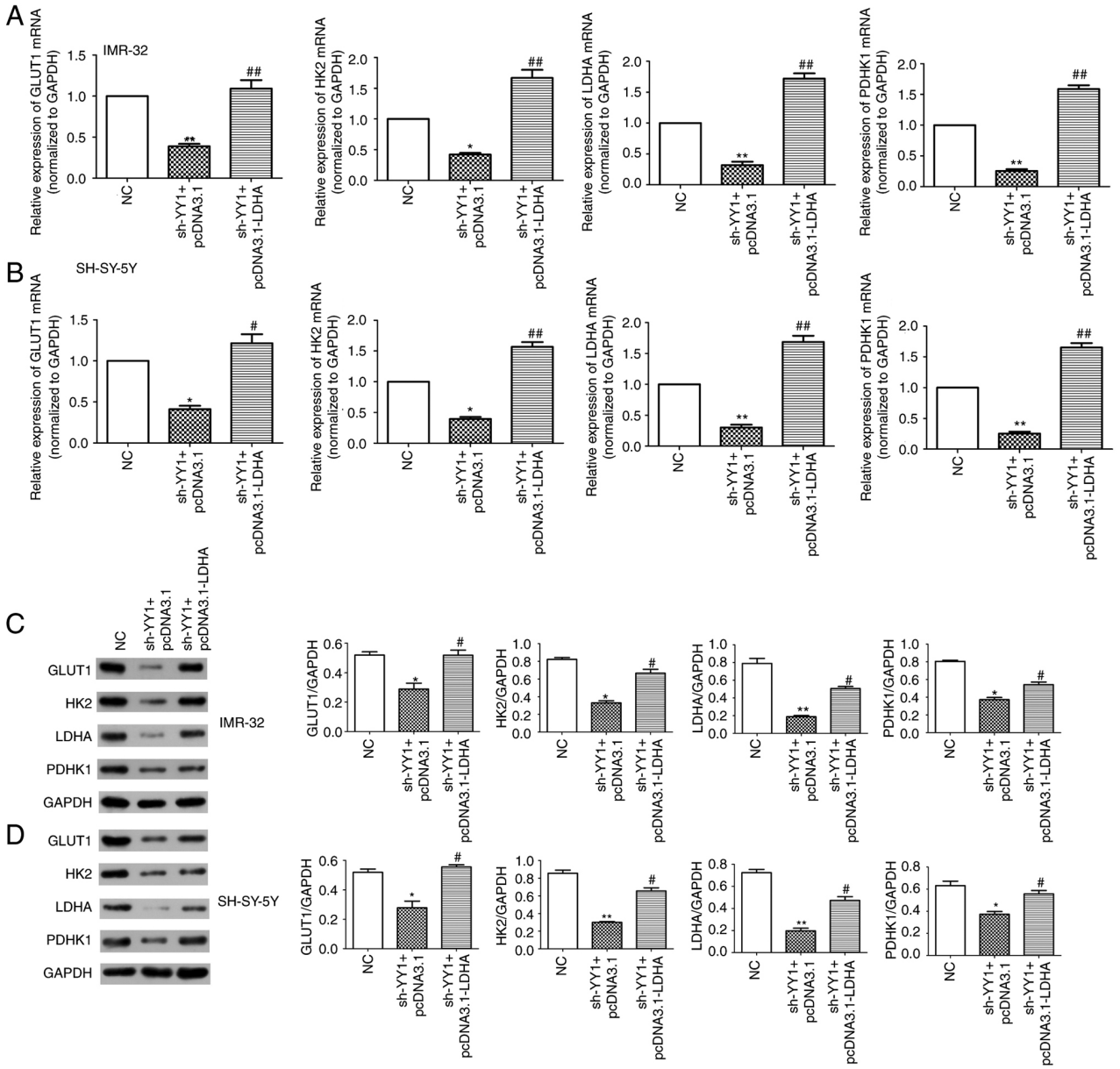

Figure 7. Overexpression of LDHA can reverse the regulatory effect of sh-YY1 on the expression of aerobic glycolytic associated molecules in neuroblastoma cells. Reverse transcription-quantitative PCR was performed to detect the mRNA expression levels of GLUT1, HK-2, LDHA and PDHK1 in (A) IMR-32 and (B) SH-SY-5Y cells. Western blotting assay was used to detect the expression levels of GLUT1, HK-2, LDHA and PDHK1 in (C) IMR-32 and (D) SH-SY-5Y cells. *P<0.05 and **P<0.01 vs. NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. sh-YY1 + pcDNA3.1 group. YY1, yin yang 1; NC, negative control; sh, short hairpin; GLUT1, glucose transporter 1; HK-2, hexokinase 2; LDHA, lactate dehydrogenase A; PDHK1, pyruvate dehydrogenase.

to relieve malignant proliferation and invasion of cancer cells (27,28). GLUT1, a member of the GLUT family, the glucose transporter is an important protein that regulates intracellular glycolytic flux (29). Li *et al* (30) demonstrated that downregulation of HK-2-mediated glycolysis can inhibit the malignant progression of colorectal cancer. PDHK1 catalyzes glucose phosphorylation and inactivates PDH, the key enzyme for pyruvate to enter the citric acid cycle. PDHK1 is also overexpressed in cancer cells, preferentially degrading pyruvate to lactate for aerobic glycolysis (31). LDHA is involved in regulating the breakdown of glucose to lactate, providing cells with a continuous supply of energy and metabolic requirements (32). Hua *et al* (33) revealed that miR-142-3p inhibits aerobic glycolysis and cell proliferation in hepatocellular carcinoma by targeting LDHA. Han *et al* (34) reported that miR-383 regulates LDHA expression in ovarian cancer cells, thereby stunting glycolysis, cell proliferation and invasion. The present study revealed that overexpression of YY1 promoted glucose uptake and lactate accumulation, and YY1 upregulated the protein expression levels of LDHA, GLUT1, HK-2 and PDHK1. It is hypothesized that YY1 targeting binding to LDHA to promote aerobic glycolysis may be its potential molecular mechanism to accelerate neuroblastoma proliferation.

In summary, the current study reported a significant upregulation of the transcription factor YY1 in neuroblastoma, suggesting its possible involvement in the malignant development of neuroblastoma cells. In-depth studies confirmed that YY1 can target and regulate LDHA and promote aerobic glycolysis, which may be closely associated with its promotion

of malignant proliferation. The present study provided a novel therapeutic strategy for the clinical treatment of neuroblastoma, and YY1 could be a potential target.

In conclusion, the present study explained the regulatory mechanism of YY1 in neuroblastoma, mainly involving aerobic glycolysis and cell proliferation. YY1 may become a novel drug target for the treatment of neuroblastoma, which may provide a new clinical treatment strategy for neuroblastoma. However, the present study did not perform *in vivo* experimental verification. Further studies will explore the association between the regulatory mechanism of YY1 on glioma proliferation and aerobic glycolysis in the mouse model.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QW conceptualized the study, designed methodology and wrote the original manuscript. WF designed methodology and performed data analysis. BL performed cell experiments, transfections and visualized the data. BH used software for data analysis and performed the literature search. ZJ performed data analysis. CL performed the acquisition of data, data analysis and reviewed and edited the manuscript. All authors read and approved the final manuscript. QW and WF confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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