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The Role of NCOA4 in Glioma Progression and TMZ Sensitivity

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Abstract

Purpose: NCOA4 (Nuclear Receptor Coactivator 4) is known to be involved in ferroptosis. However, its function and molecular mechanisms in gliomas are still unclear.

Methods: We identified the NCOA4 expression in glioma cells and glioma specimens by Western blot (WB). Overexpression NCOA4 glioma cell lines were induced by recombinant adenovirus of NCOA4, and down-regulation NCOA4 glioma cell lines were induced by siRNA of NCOA4. GSH Detection Kit and DCFDA assay were used to detect cell GSH and ROS levels. CCK-8 and Transwell assays were used to detect cell proliferation and the sensitivity of glioma cells to temozolomide (TMZ).

Results: We identified that NCOA4 was downregulated in glioma cell lines and specimens. NCOA4 overexpression inhibited the glioma cells proliferation, while NCOA4 knockdown promoted the glioma cells proliferation. NCOA4 could enhance

sensitivity of glioma cells to temozolomide (TMZ) treatment. And NCOA4 overexpression inhibited GSH and promotes ROS accumulation in gliomas.

Conclusion: NCOA4 was downregulated in gliomas and inhibited the gliomas proliferation. NCOA4 sensitized glioma cells to TMZ

Keywords: NCOA4, Glioma Progression, PTCH1; TMZ Sensitivity; Tissue Microarray

Introduction

Gliomas are the most dangerous central nervous system tumors [1]. Despite the rapid development of related diagnostic and therapeutic technologies, the prognosis of glioma patients remains poor, the median survival is only 10-15 months [2,3]. The introduction of alkylating agent temozolomide (TMZ) has improved the survival of glioma patients, while resistance to TMZ treatment is also one of the important reasons for poor outcome of glioma patients. Therefore, research on glioma mainly focuses on new mechanisms and treatment methods, but the molecular mechanism of glioma and solutions for TMZ resistance remains limited.

NCOA4 was a coactivator for a variety of nuclear receptors, like aryl hydrocarbons, steroid hormones, thyroid hormones, and vitamin D and A [4]. NCOA4 is also associated with cellular ferroptosis which can stimulate iron release [5]. There are few reports about NCOA4 in gliomas. The role of NCOA4 on glioma proliferation and TMZ sensitivity in glioma remains unclear.

Methods and Materials

Reagents and Consumables

Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM) and Tyrisin (0.25% EDTA) were purchased from Gibico Life Technologies (Geand land, New York, USA). Antibodies for β-actin was from proteintech (Wuhan, China). Antibodies for NCOA4 supplied by Abcam (Cambridge, UK). Protease inhibitor, RIPA, dimethylsulfoxide (DMSO), Lysis buffer, SDS-PAGE loading buffer were supplied by Solarbio Life Science (Beijing, China). PVDF membranes were from Millipore (Bedford, MA, USA). TMZ and Cell counting kit-8 (CCK-8) was from Dojindo Molecular Technologies, Inc (Kumamoto, Japan). The siRNAs were purchased from Aibosi Biotechnology (Shanghai, China). Recombinant adenovirus of NCOA4 was purchased from Jikai Biotechnology (Shanghai, China).

Glioma Cell Lines and Cell Culture

The human astrocyte (AS), A172, U87, 23N, SHG44, T98G, LN18, TJ899, B2-17, U251 and SNB19 glioma cell lines were preserved by Neuro-Oncology Laboratory, Tianjin Institute of Neurology. All cell lines were cultured in 37℃ 5% CO2 incubator and DMEM containing 10% FBS (fetal bovine serum).

Glioma Specimens

28 glioma specimens, including Grade I: 4, Grade II: 7, Grade III: 7, Grade IV: 10, and 3 nontumorous brain tissues were obtained from Department of Neurosurgery, Tianjin Huanhu Hospital during 2021~2022. The glioma tissues and nontumorous tissues were treated with 500ul RIPA, incubated on ice for 15 min, centrifuged at 12000rpm×15min, the supernatant was taken, appropriate 4× SDS--PAGE loading buffer, water bath boiled 5min and reserved at -20℃ for western blot(WB).

Cell Transfection and Protein Detection

When the cultured cells density reached 50%-70%, the siRNA-NCOA4 was transfected into LN18 and T98G using Lipofectamine 3000, and the Adv- NCOA4 (recombinant adenovirus of NCOA4) was transfected into LN18 and T98G cells using ADV helper reagent, 48h after transfection, proteins were extracted using RIPA Lysis buffer (PMSF, 1:100).

Proliferation Analysis

For CCK-8 assay, glioma cells were inoculated into 96-well plates and proliferation capacity were detected using CCK-8 kit according to the manufacturer's instructions.

Measurement of GSH

Glutathione levels measured with Reduced GSH Detection Kit (Nanjing Jincheng, China)

ROS Measurement

ROS analysis was conducted using 2 ,7 -Dichlorofluorescein diacetate (DCFH-DA) (35845, Sigma). Cells from each group were digested with EDTA-free trypsin, washed three times, and then treated with DCFH-DA at a final concentration of 10μM. Subsequently, cells were incubated for 30 min and gently mixed by inverting every 3–5min. The fluorescence intensity was measured using a CytoFLEX Research Flow Cytometry instrument (Beckman Coulter) at 488nm excitation and 525nm emission wavelengths. Data were analyzed using FlowJo 10 software.

Statistical Analysis

Statistical analysis were carried out using SPSS Statistics 25.0 of ANOVA analysis or Student's test. *P < 0.05 was considered statistical significance. The data were presented as mean ±SD (standard deviation) and the graphs were drawn by GraphPad Prism 6.0 (GraphPad Software, San Diego, California, USA).

Results

Downregulation of NCOA4 Expression in Gliomas

We examined the expression of NCOA4 in glioma cell lines and specimens by WB. NCOA4 were downregulated in most glioma cell lines compared with that in astrocytes, a significant decrease in LN18, SHG44, A172, T98G, U251 and SNB19 cell lines, and a slight decrease in 23N, U87, TJ899 and B2-17 cell lines (Figure1A). We examined the expression of NCOA4 in 28 glioma specimens and 3 nontumorous tissues by WB. Compared with nontumorous tissue, NCOA4 is downregulated in glioma specimens and negatively correlated with tumor grades (Figure1B).

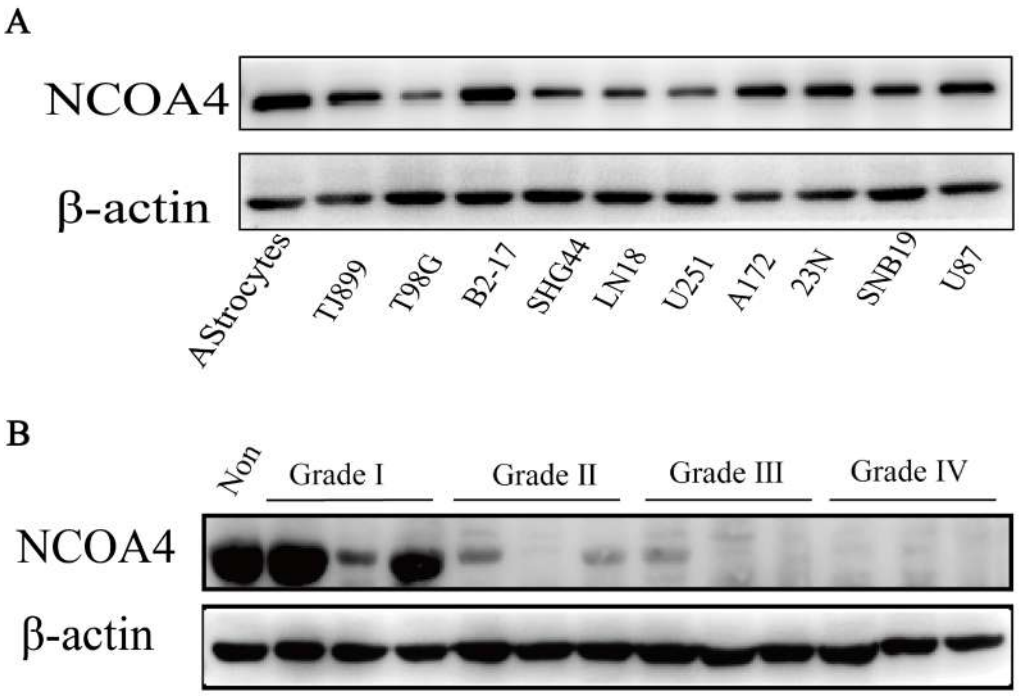

Figure 1: NCOA4 expression is downregulated in glioma cell lines and specimens. (A)The protein level of NCOA4 was analyzed in glioma cell lines (A172, U87, 23N, SHG44, T98G, LN18, TJ899, B2-17, U251 and SNB19) and astrocytes. (B) The protein expression of NCOA4 was analyzed in glioma tissues and nontumorous brain tissues.

NCOA4 Inhibits the Progression of Gliomas

Adv-NCOA4 was applied to overexpress the expression of NCOA4. NCOA4 overexpression suppressed the proliferation of LN18 and T98G cells (Figure 2A). There was a significant difference between the NCOA4 overexpression group and control group since the second day (P<0.05). SiRNA-NCOA4 was transfected to knock down NCOA4 in LN18 and T98G cells, NCOA4 downregulation promoted glioma cell lines growth. the significant difference also could be observed on the 2nd day in LN18 cells and 3rd day in T98G cells (Figure2B). These results suggested that NACOA4 suppresses the progression of gliomas.

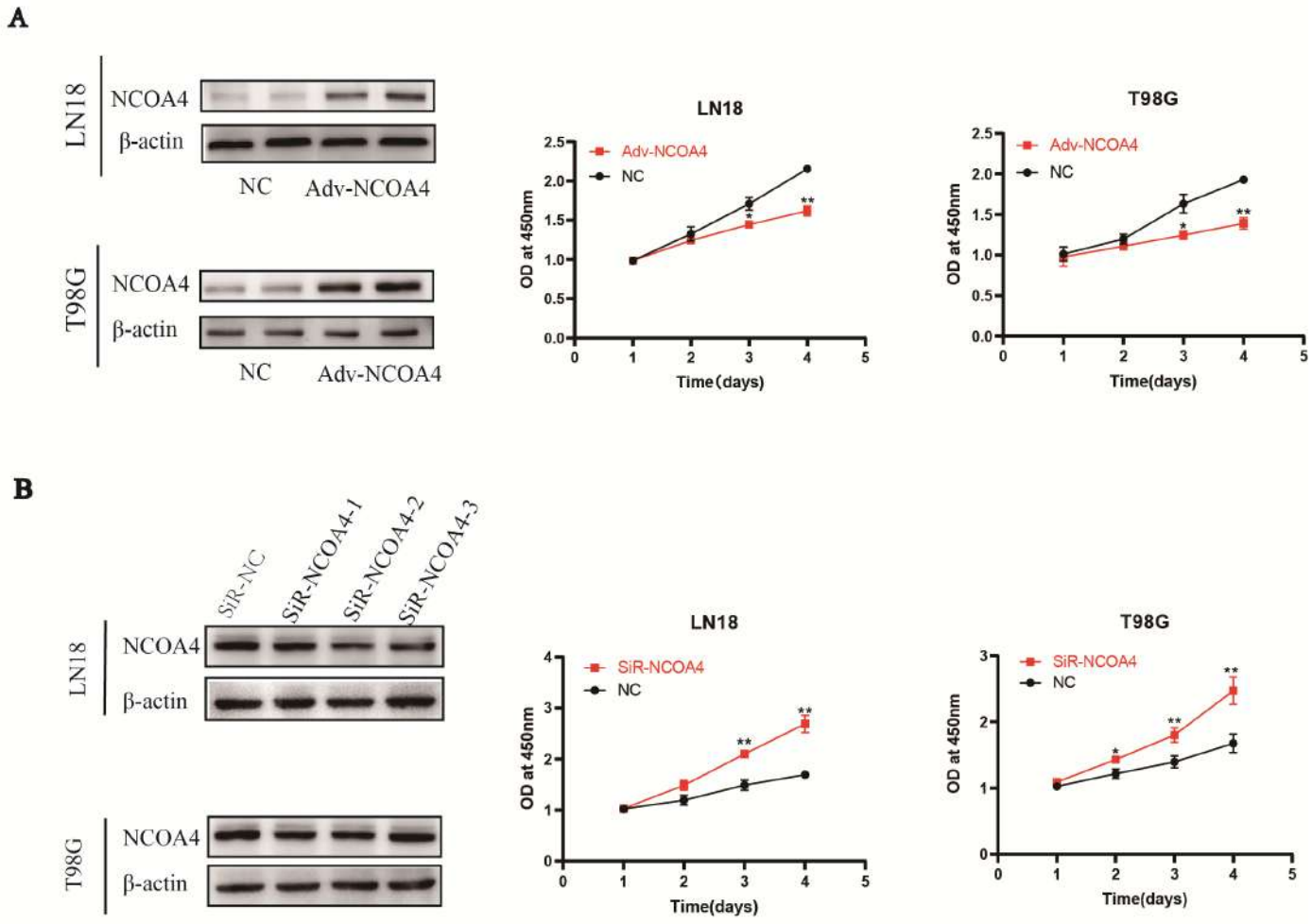

Figure 2: NCOA4 suppresses the growth of glioma cells. (A,B) NCOA4 knockdown and overexpression in LN18 and T98G cells. CCK-8 assays after NCOA4 knockdown and overexpression in glioma cell lines. (*, P<0.05; **, P<0.01 compared with control group.)

NCOA4 Enhances the TMZ Sensitivity in Glioma Cells

To investigate whether NCOA4 affects TMZ sensitivity, we detected the proliferation and invasion ability of glioma cells treated with TMZ and NCOA4 overexpression+TMZ,. CCK-8 and transwell assay demonstrated that NCOA4 overexpression promotes the growth inhibition rate and invasion inhibition of TMZ in glioma cells, indicated that NCOA4 sensitized glioma cells to TMZ (Figure3A,3B).

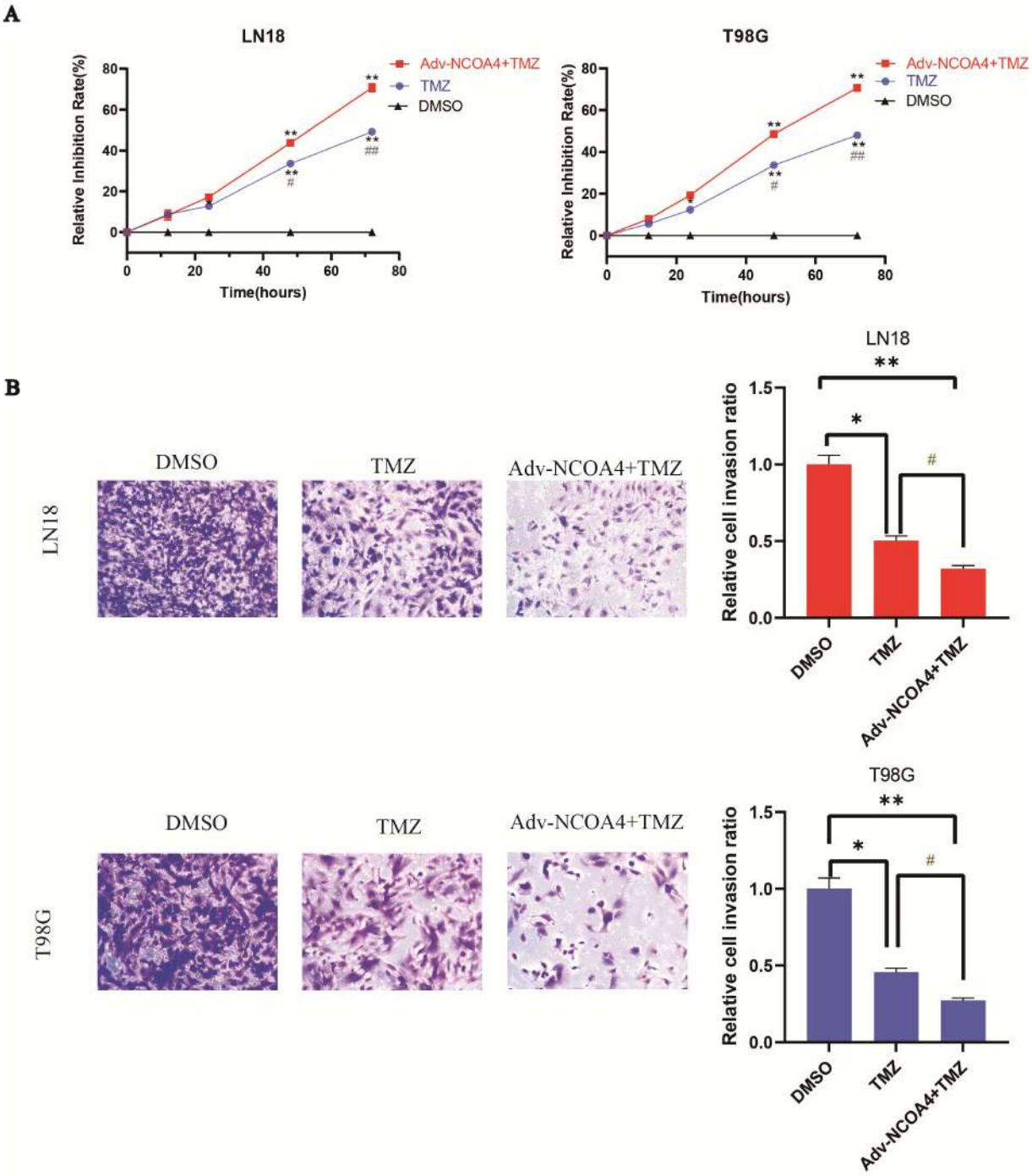

Figure 3: NCOA4 promotes TMZ-mediated inhibition of proliferation and invasion ability in glioma cells. (A) CCK-8 assays of DMSO group, TMZ group and Adv-NCOA4+TMZ group in LN18 and T98G cell lines. (B) Transwell assays of DMSO group, TMZ group and Adv-NCOA4+TMZ group in LN18 and T98G cell lines. *, P<0.05; **, P<0.01, compared with the DMSO group. #, P<0.05; ##, P<0.01, compared with the Adv-NCOA4

NCOA4 Inhibits GSH and Promotes ROS Accumulation in Gliomas

We tried to find the specific mechanism by how NCOA4 sensitizes TMZ. TMZ sensitivity is associated with GSH and ROS. We found NCOA4 overexpression group exhibited significantly higher basal ROS levels and lower GSH levels than control group (Figure4A,4B).

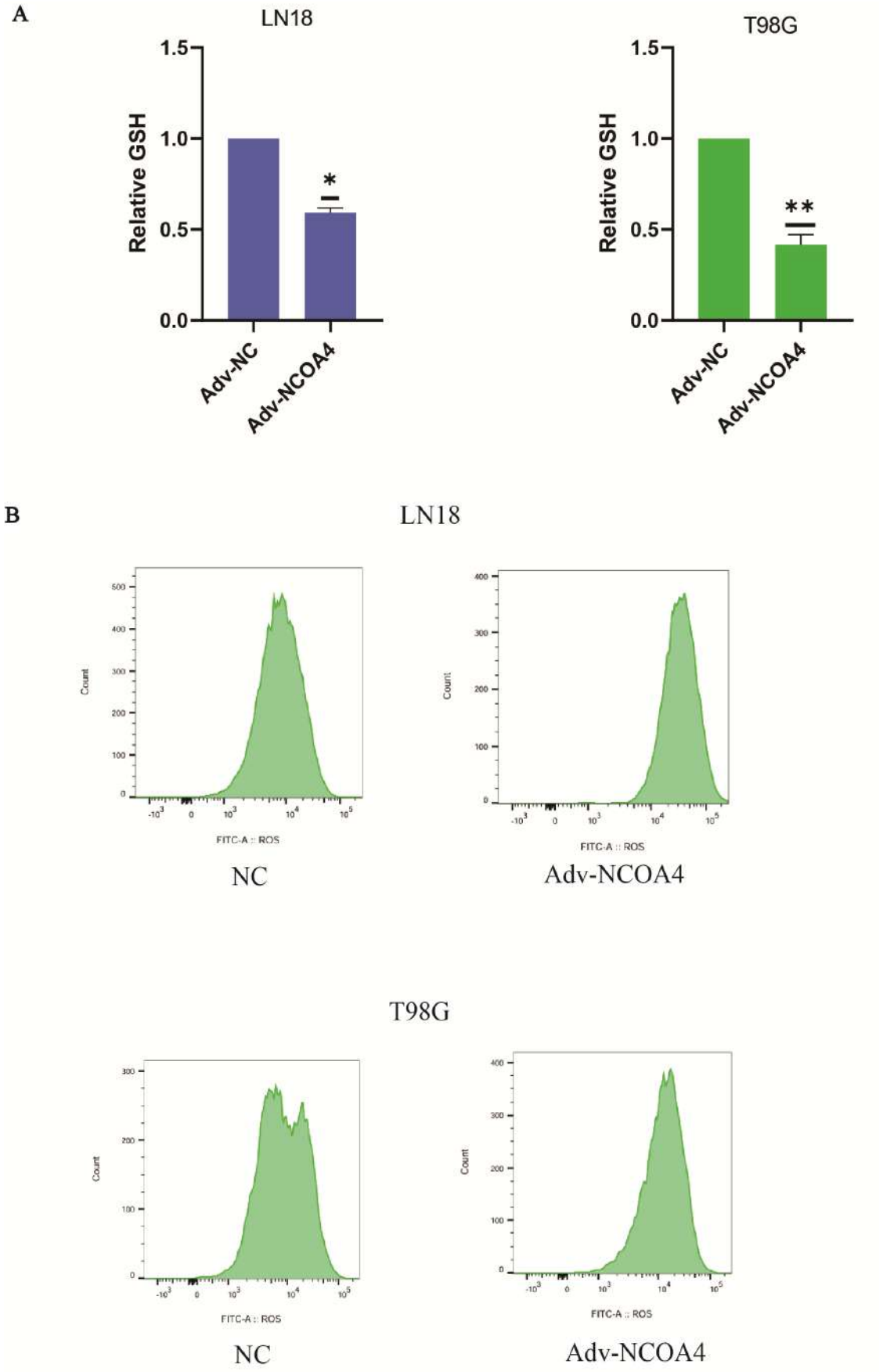

Figure 4: NCOA4 inhibits GSH and promotes ROS accumulation (A) Relative GSH levels were assessed using GSH Detection Kit. (*, P<0.05; **, P<0.01 compared with control group.) (B) ROS levels of were assessed using the DCFDA assay.

Discussion

With the advancement of technology, there are more and more cognitive and diagnostic methods for glioma. But the role of NCOA4 in gliomas remains unclear. We detect the expression of NCOA4 in glioma cells by WB, NCOA4 was downregulated in glioma compared with non-tumorous brain tissues, and negatively correlated with glioma grades. To identify the effect of NCOA4 on the growth of glioma, NOCOA4 was overexpressed in glioma cells, NCOA4 overexpression significantly inhibited glioma cells proliferation. While proliferation were promoted when NCOA4 was knocked down. These results demonstrated that NCOA4 might play an inhibitory role in glioma progression.

TMZ is commonly used and primary chemotherapeutic drug for glioma chemotherapy. Resistance to TMZ is an important challenge in the treatment of gliomas. We tried to explore the relation between NCOA4 and TMZ and demonstrated that NCOA4 could sensitize glioma cells to TMZ. Some studies reported that an increase in ROS enhanced glioma cell sensitivity to TMZ and reduced GSH concentration could inhibit TMZ resistance [6,7]. We found that ROS levels were higher and GSH levels were lower in NCOA4 overexpression group compared with control group. NCOA4 may enhanced TMZ sensitivity through regulating ROS and GSH.

In summary, the expression of NCOA4 was downregulated in glioma, negatively correlated with glioma grades NCOA4 suppressed glioma progression and increased TMZ sensitivity. These results suggested NCOA4 might be a promising therapeutic target for glioma.

Funding

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Declaration of Interests

The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards

This study was carried out in accordance with the principles of the Ethics Committee of Tianjin Huanhu Hospital.

Author Contributions

L.K. and W.H wrote the main manuscript text and prepared all figures. Q.T. W.G. and Z.A. helped L.K.to collect datas and experiment. J.Z. and T.X reviewed and modified the article. W.H and T.X. provided the Fundings. All authors have reviewed and approved the final version of this manuscript.

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