

Original Article

Regulation and molecular mechanism of adiponectin on the proliferation, apoptosis, autophagy, and chemosensitivity of LN18 Glioma cell line

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Abstract



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This study aimed to explore the regulatory effects and associated mechanisms of adiponectin on apoptosis and proliferation in the LN18 glioma cell line through the AMPK and Akt signaling pathways. Additionally, we sought to elucidate the impact of adiponectin on the chemosensitivity of the LN18 glioma cell line to temozolomide (TMZ). The proliferation rate of glioma cells treated with adiponectin was assessed using the cholecystokinin (CCK8) assay. The Western blot analysis was employed to assess the expression of p-Akt, p-AMPK, p-mTOR, cleaved caspase3, Bax, Cyclin D1, and Cyclin B1 following adiponectin treatment. Cell apoptosis was quantified using AnnexinV/PI flow cytometry, while changes in the cell cycle were detected using PI staining flow cytometry. The findings revealed that adiponectin upregulates p-AMPK expression and downregulates p-mTOR expression in the PTEN wild-type glioma cell line LN18, with no discernible effect on p-Akt expression. Moreover, adiponectin inhibits the proliferation rate of the PTEN wild-type glioma cell line LN18, enhances the expression of cleaved caspase3 and Bax, and significantly elevates the apoptosis rate, as evidenced by AnnexinV/PI flow cytometry. Adiponectin was observed to suppress the expression of Cyclin D1 and Cyclin B1, increase the number of cells in the G1 phase, and promote autophagy. Additionally, adiponectin augments the expression of Beclin1 and the ratio of LC3II/I in the PTEN wild-type glioma cell line LN18, while decreasing p62 expression. In conclusion, this study posits that adiponectin holds therapeutic promise for glioma treatment. Furthermore, adiponectin enhances the inhibitory effect of TMZ on the proliferation rate of LN18 cells when treated with 0.1 mM and 1 mM TMZ. These results collectively suggest that adiponectin impedes proliferation, encourages apoptosis and autophagy in the LN18 glioma cell line, and heightens its sensitivity to the chemotherapeutic drug TMZ.

Keywords: Adiponectin, LN18, Glioma, Apoptosis, Autophagy, Cell Cycle, Chemosensitivity

1. Introduction

Gliomas represent the most prevalent primary malignant brain tumors among adults, characterized by a dismal prognosis. Lifestyle has recently emerged as a significant risk factor in the development of primary glial malignancies [1,2]. While treatment approaches involving surgical resection, post-operative radiotherapy, and TMZ-based chemotherapy have shown success for gliomas [3,4], there remains a critical need for the exploration of natural and non-toxic compounds to augment the effectiveness of glioma treatment.

Adiponectin, an adipocyte-secreted adipokine with diverse metabolic effects, has been implicated in various malignancies, with reduced levels linked to their development and progression [5-7]. Known for its advantageous metabolic effects, adiponectin's AMPK pathway plays a crucial role in regulating cell autophagy [8,9]. Alterations in adiponectin expression and receptors significantly impact diseases such as cancer, diabetes, and liver injury [10-12]. Additionally, other adipokines, like AEBP1, have demonstrated inhibitory effects on human glioma

cell proliferation, invasion, and early apoptosis induction [13-15]. Recent studies have indicated that adiponectin can enhance chemotherapy sensitivity in specific cancer treatments [16]. Recognized for its potential as a treatment target in fibrotic diseases, liver injuries, and cancer cell autophagy, adiponectin's role in inhibiting breast cancer growth and inducing apoptosis has also been suggested [17,18]. Although the anti-cancer mechanism of adiponectin is associated with the AMPK pathway [19,20], its role in gliomas remains unclear. Therefore, it is crucial to further investigate the regulatory effects and molecular mechanisms of adiponectin on the proliferation, apoptosis, autophagy, and chemotherapy sensitivity of the LN18 glioma cell line.

Our study aims to explore the regulatory effects and mechanisms of adiponectin on apoptosis and proliferation of the LN18 glioma cell line through AMPK and Akt signaling pathways. Additionally, it aims to clarify the impact of adiponectin on the sensitivity of the LN18 glioma cell line to TMZ chemotherapy.

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2. Materials and methods

2.1. Cell line

Gliomas cell lines LN18, U87MG, and U251 cells were obtained from the American Type Culture Collection (ATCC), thawed from early-passage stocks kept in liquid nitrogen vapor as required, and cultured according to the supplier's instructions.

Human recombinant adiponectin (R&D Systems, USA), Adiponectin (0, 0.1 µg/mL, 0.5 µg/mL, 1 µg/mL, 3 µg/mL, 10 µg/mL) is to be mixed with cell culture medium (90% DMEM high-glucose medium + 10% fetal bovine serum + bispecific antibiotic (100 U/mL) at the required concentrations and co-incubated in a constant temperature cell culture incubator at 37°C, with 5% CO₂ and 95% humidity, for durations of 0 h, 1 h, 6 h, 12 h, 24 h, and 48 h.

2.2. Cell and passage

The U87MG cells were incubated at room temperature for approximately 30 s, while the U251 cells were incubated at 37°C for about 1 min, The LN18 cells underwent a similar incubation period at room temperature for about 1 min.

Upon observing a change in the cells to a spherical shape, 2 mL of high-glucose DMEM medium containing 10% fetal bovine serum was added. The cells were subsequently centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. Finally, the cells were resuspended in 4 mL of high-glucose DMEM medium containing fetal bovine serum. Following this, an additional 4 mL of high-glucose DMEM medium containing 10% fetal bovine serum was introduced. The resulting cell suspension, with a density of 3-7×10⁵ cells/mL, was sequentially seeded into new cell culture flasks. These flasks were then placed in a cell culture incubator set at 37°C with a constant temperature, 5% CO₂, and 95% humidity.

2.3. Cell recovery

Upon retrieval from liquid nitrogen, the cryopreserved cells should be promptly immersed in a 37°C water bath for 1 min to facilitate thawing. Subsequently, transfer the cell suspension to a sterile 15 mL centrifuge tube using a pipette and add 10 mL of cell culture medium. Centrifuge the tube at 1000 rpm for 5 min utilizing a high-speed centrifuge. Discard the supernatant and resuspend the cells in 4-5 mL of high-glucose DMEM medium containing 10% fetal bovine serum. The cells should then be seeded

into pre-prepared cell culture flasks and incubated in the cell culture incubator. On the following day, the medium should be replaced with fresh cell culture medium and subsequently changed every 2-3 days.

2.4. Assessment of adiponectin-induced alterations in Glioma cell proliferation via CCK8 Assay

10 µL of cell suspension was combined with 10 µL of 0.3% Trypan Blue and introduced into an electronic cell counting plate for enumeration. U87MG cells were plated at a density of 7×10⁴ cells/well in a 24-well plate, while LN18 and U251 cells were plated at a density of 5×10⁴ cells/well in a 24-well plate. The plate was gently tapped to ensure uniform cell distribution, and control and blank wells were included, with 1500 µL of culture medium added to each well. Adiponectin was administered at predetermined concentrations and specific time points.

At the time of measurement, 1000 µL of culture medium was aspirated from each well, followed by the addition of 50 µL of CCK8 solution. The plate was then incubated at 37°C for 1 h, and the OD₄₅₀ value was measured using an enzyme-linked immunosorbent assay reader. The cell proliferation rate was calculated using the following formula.

$$\text{cell survival rate} = \frac{D_{\text{case}} - D_{\text{n}}}{D_{\text{con}} - D_{\text{n}}}$$

2.5. Western blot

Prepare the protein extraction working solution according to the instructions provided by the protein extraction kit. Add the RIPA cell lysis solution and incubate for 3 min. Perform ultrasonic fragmentation with 10 s intervals for a total of 40 s. Vortex the solution for 10 s with 10 s intervals for a total of 1 min. Centrifuge the solution at 12,000 rpm for 26 min, and store the supernatant at -80°C for future use. Determine the total protein content using the BCA protein quantification kit. For protein sample preparation, add 25% of the sample volume of 5×protein sampling buffer and stir well. Then, incubate in an open water bath for 10 min. After SDS-PAGE gel electrophoresis and separation of the target proteins, transfer them to the NC membrane. Seal the membrane and add the diluted primary antibody (the dilution concentration of the primary antibody can be found in Table 1). Incubate overnight at 4°C. Discard the primary antibody and add TBST solution. After cleaning

Table 1. Dilution concentration of the primary antibody for Western blot.

Antibody	Dilution concentration
Rabbit anti-adiponectin receptor 1 (AdipoR1)	1:1000
Goat anti-adiponectin receptor 2 (AdipoR2)	1:500
Rabbit anti-Akt	1:1000
Rabbit anti-p-Akt (Thr308)	1:500
Rabbit anti-p-Akt (Ser473)	1:500
Rabbit anti-Caspase-3	1:1000
Rabbit anti-Cleaved Caspase-3	1:500
Rabbit anti-Bax	1:1000
Rabbit anti-Cyclin D1	1:1000
Rabbit anti-p21 (phospho T145)	1:500
Rabbit anti-GAPDH	1:4000
Rabbit anti-β-actin	1:4000

the NC membrane, add the secondary antibody (diluted with skimmed milk powder at a ratio of 1:500). Develop the sample using the ECL luminescence kit method, ensuring a fixation time between 6-9 min. Analyze the grey value of the specific bands using Genetools software. The expression levels of p-Akt, p-AMPK, p-mTOR proteins, apoptosis-related proteins (cleaved caspase-3 and Bax), and cell cycle-related proteins (Cyclin D1 and Cyclin B1) in the cells were assessed through Western blot analysis.

2.6. Assessment of cell apoptosis levels through AnnexinV/PI flow cytometry analysis

To harvest cells, detach adherent cells and transfer them to a 10 mL centrifuge tube as a single-cell suspension. The cell number for each sample should range approximately from 1×10^6 - 5×10^6 . Centrifuge the cells at 1000 rpm for 6 min using a low-speed centrifuge and discard the supernatant. Incubate the collected cells with incubation buffer and wash them with a low-speed centrifuge at 1000 rpm for 6 min. Finally, resuspend the cells in 120 μ L of labeling solution and incubate them in the dark at room temperature for 14 min.

Centrifuge the sample at 1000 rpm for 6 min and wash the cells once with incubation buffer. Mix the sample cells with the fluorescent working solution and incubate at 4°C for 22 min. Analyze the AnnexinV/PI double-stained samples using a flow cytometer with an excitation wavelength of 488 nm, $\lambda_{\text{FITC}} = 515$ nm, and $\lambda_{\text{PI}} > 560$ nm.

The third quadrant of the bivariate scatter plot represents viable cells, where both FITC and PI staining are low or negative. The second quadrant represents necrotic cells, where both FITC and PI staining are positive. The fourth quadrant represents early apoptotic cells, which are FITC-positive and PI-negative. The first quadrant represents late apoptotic cells, which are PI-positive only. The apoptotic rate is calculated by adding the percentages of cells in the first and fourth quadrants.

2.7. Cell cycle analysis by Propidium Iodide (PI) staining using Flow Cytometry

Cells in logarithmic growth phase were harvested, digested, and resuspended in $1 \times \text{PBS}$ at 4°C. Subsequently, the cells were centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. Following that, 2 mL of pre-cooled 75% ethanol was added to fix the cells in the wells and left overnight. The cells were then centrifuged at 2000 rpm for 5 min at room temperature, and the supernatant was discarded. The cells were suspended in 2 mL of PBS and centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and the cells were re-suspended in 2 mL of PBS.

Subsequently, 100 μ L of a 5 mg/mL RNase solution was added to the cells and incubated at 37°C for 30 min to remove RNA. After the RNase treatment, 100 μ L of a 1 mg/mL PI staining solution (50 μ g/mL final concentration) was added to the cells and incubated in the dark for 30 min. The cells stained with PI, along with digested RNA and PI, were filtered through a 300-mesh nylon mesh and resuspended. The cell cycle was analyzed using a flow cytometer.

2.8. Statistical analysis

The means of the quantitative data were expressed as mean \pm standard deviation (SD). Data analysis was conduc-

ted using Statistic Package for Social Science (SPSS) 25.0 statistical software (IBM, Armonk, NY, USA). When the data exhibited normal distribution and homogeneity of variance, a one-way analysis of variance (ANOVA) was employed to compare mean values across multiple groups. Pairwise comparisons between groups were performed using the least significant difference (LSD) test. In cases where variance was not homogeneous, the Kruskal-Wallis non-parametric test was utilized, with a significance level set at $P < 0.05$.

3. Results

3.1. The expression of adiponectin receptors in LN18 glioma cells

We employed immunoblotting to assess the expression of adiponectin receptors in the LN18 glioma cell line and observed the presence of both adiponectin receptor 1 and adiponectin receptor 2 (Figure 1).

3.2. The impact of adiponectin on the proliferation rate of LN18 cells

CCK8 was employed to assess the dose-dependence and time-dependence of adiponectin on the proliferation rate of LN18 glioma cells. The results indicated that when the concentration of adiponectin exceeded 3 μ g/mL, the cell proliferation rate in the LN18 cell line was markedly lower than that of the control group ($P < 0.001$). Additionally, there was no significant difference in the inhibitory effect on the cell proliferation rate between 10 μ g/mL adiponectin and 3 μ g/mL adiponectin ($P > 0.05$). (Figure 2).

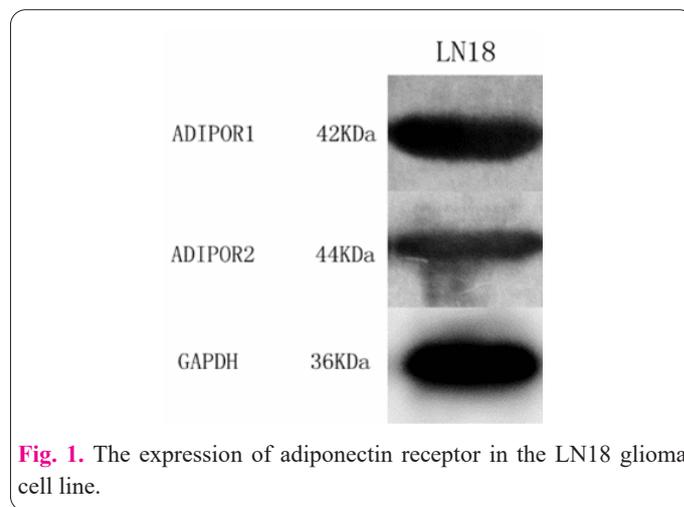


Fig. 1. The expression of adiponectin receptor in the LN18 glioma cell line.

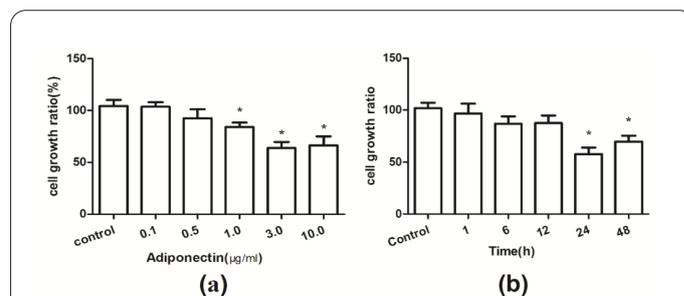


Fig. 2. Effect of adiponectin on LN18 glioma cell proliferation. (a) Dose-dependent relationship of adiponectin on the proliferation of LN18 glioma cells; (b) Time-dependent relationship of adiponectin on the proliferation of LN18 glioma cells. Compared with the control group, * $P < 0.05$.

3.3. Effect of adiponectin on Akt and AMPK signaling pathways in LN18 glioma cells

After 24 h of treatment with adiponectin on the LN18 glioma cell line, immunoblotting was used to detect the expression levels of p-Akt, p-AMPK and p-mTOR. It was found that 3 $\mu\text{g}/\text{mL}$ adiponectin could significantly increase the expression of p-AMPK. Expressed and inhibited the expression of p-mTOR (Ser2448), and the difference was statistically significant compared with the control group ($P < 0.05$). However, adiponectin failed to significantly change the expression of p-Akt, and there was no statistically significant difference between the groups ($P > 0.05$) (Figure 3).

3.4. Effect of adiponectin on apoptosis of LN18 glioma cells

Apoptosis-related protein expression was assessed via immunoblotting, revealing that treating U87MG cells with varying concentrations (0, 0.5 $\mu\text{g}/\text{mL}$, 1.0 $\mu\text{g}/\text{mL}$, 3 $\mu\text{g}/\text{mL}$) of adiponectin for 24h resulted in a significant increase in the expression of cleaved caspase-3 and Bax at an adiponectin concentration of 1.0 $\mu\text{g}/\text{mL}$. The difference between the adiponectin group and the control group was statistically significant ($P < 0.05$) (Figure 4).

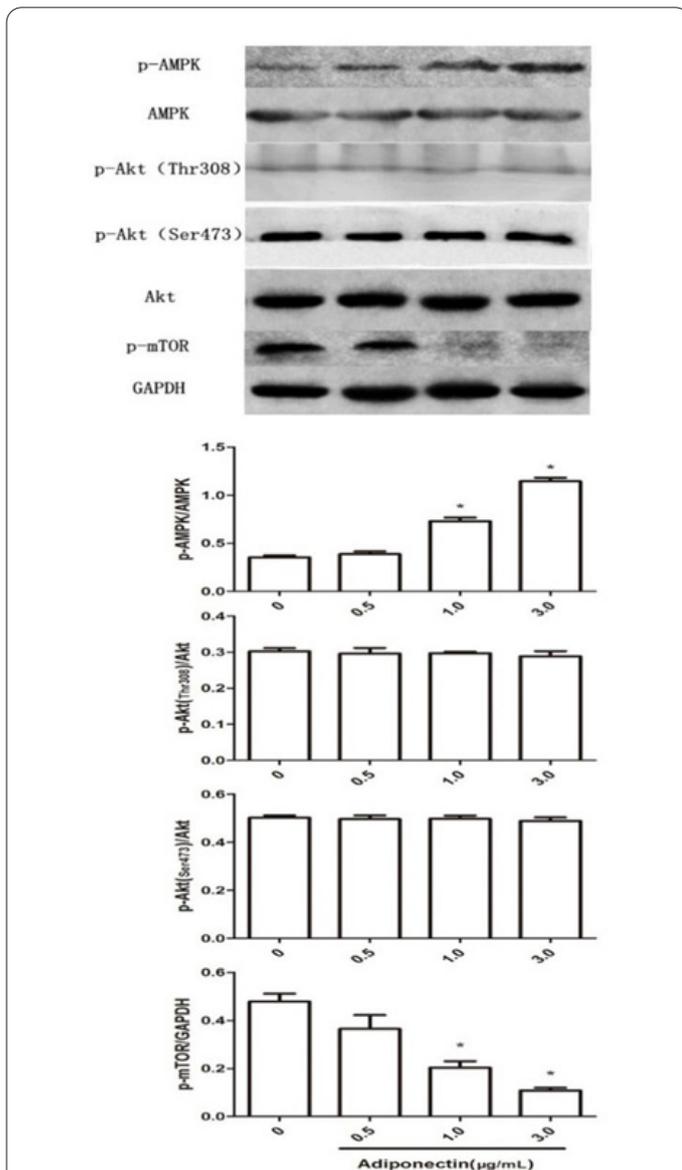


Fig. 3. Effect of adiponectin on the expression of p-AMPK, p-Akt and p-mTOR in U87MG cells. Compared with the control group, * $P < 0.05$.

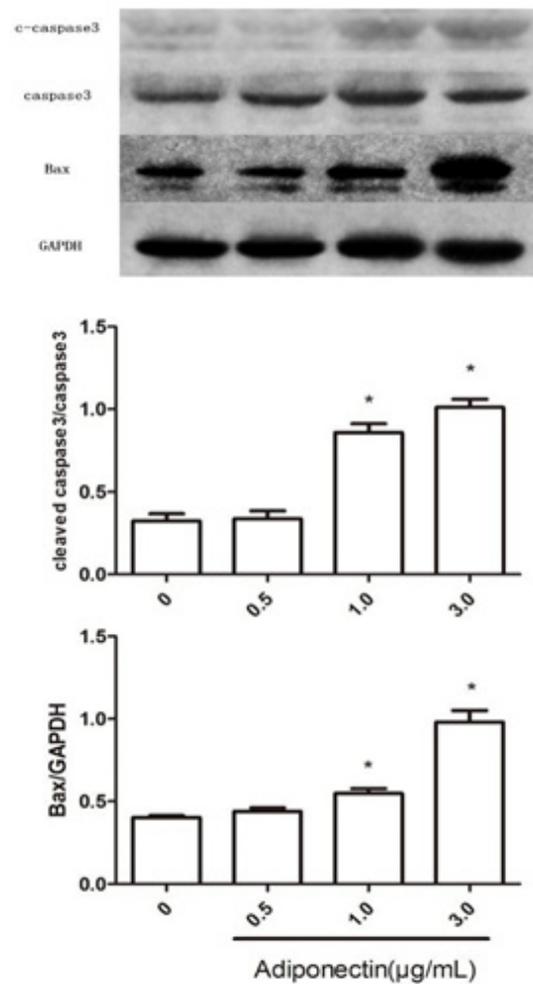


Fig. 4. Adiponectin regulates the expression of apoptosis-related proteins in LN18 cells. Compared with the control group, * $P < 0.05$.

Annexin V/PI flow cytometry was employed to assess the apoptosis level of the LN18 glioma cell line treated with 3 $\mu\text{g}/\text{mL}$ adiponectin for 24 h. It was observed that adiponectin significantly increased the apoptosis level of the LN18 cell line. Statistical analysis indicated that compared with the control group, the percentage of Q2+Q4 cells in the ADN group was significantly higher ($P < 0.05$) (Figure 5).

3.5. Effect of adiponectin on cell cycle of LN18 glioma

After 24 h of serum starvation, immunoblotting was utilized to detect the expression of proliferation-related proteins. LN18 cells were then treated with adiponectin at different concentrations (0, 0.5 $\mu\text{g}/\text{mL}$, 1.0 $\mu\text{g}/\text{mL}$, 3 $\mu\text{g}/\text{mL}$) for an additional 24h following the serum starvation period. It was observed that 1.0 $\mu\text{g}/\text{mL}$ and 3 $\mu\text{g}/\text{mL}$ adiponectin significantly inhibited the expression of Cyclin B1 and Cyclin D1. In comparison with the control group, the difference between the 1.0 $\mu\text{g}/\text{mL}$ adiponectin group and the 3 $\mu\text{g}/\text{mL}$ adiponectin group was statistically significant ($P < 0.05$) (Figure 6).

After 24 h of serum starvation, LN18 cells were treated with 3 $\mu\text{g}/\text{mL}$ adiponectin for an additional 24 h, and their cell cycle was assessed by flow cytometry with PI staining. It was observed that adiponectin significantly increased the number of cells in the G1 phase ($P < 0.05$), while the number of cells in the S phase decreased significantly (Figure 7).

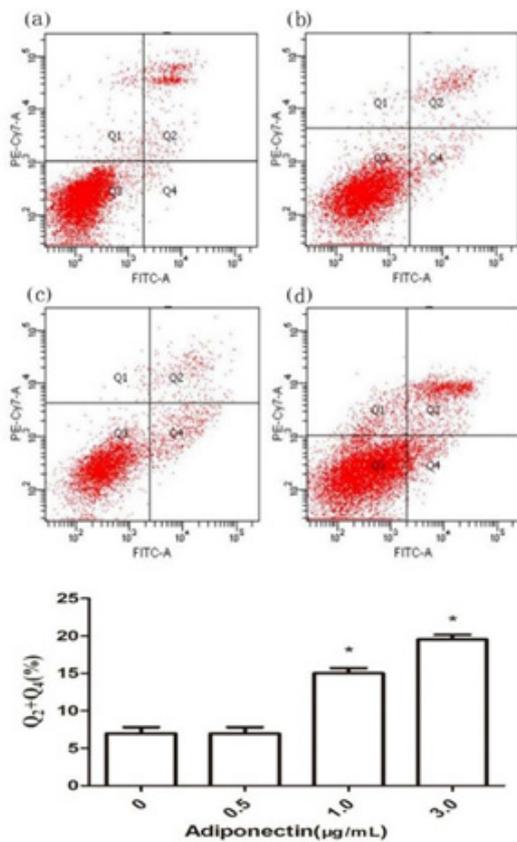


Fig. 5. Effect of adiponectin on apoptosis of LN18 glioma cells. (a) Control group; (b) Adiponectin 0.5 µg/mL; (c) Adiponectin 1.0 µg/mL; (d) Adiponectin 3.0 µg/mL. Compared with the control group, *P<0.05.

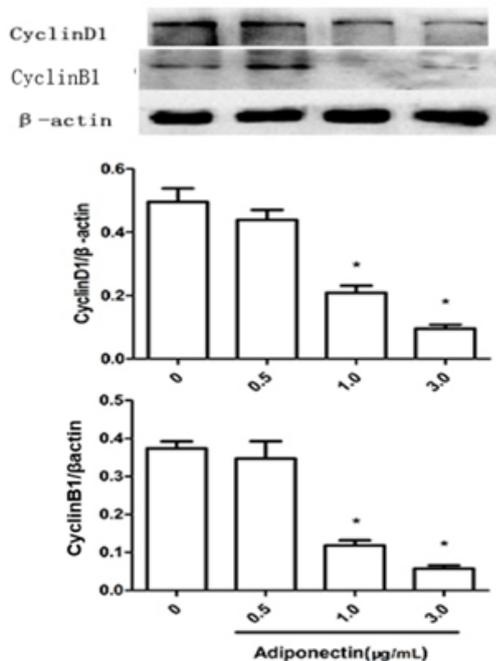


Fig. 6. Adiponectin regulates the expression of LN18 cell cycle-related proteins. Compared with the control group, *P<0.05.

3.6. Effect of adiponectin on autophagy level of LN18 glioma cells

The results obtained from immunoblotting to detect autophagy-related proteins revealed that, after treatment of LN18 cells with 1 µg/mL and 3 µg/mL adiponectin for 24

h, the expression of Beclin1 protein increased compared to the control group. Additionally, the expression level of LC3II relative to LC3I significantly increased, and there was a notable increase in the consumption of p62 protein. Gray value analysis demonstrated statistically significant differences in the expression levels of autophagy-related proteins (Beclin1, LC3II/I, p62) between the 1 µg/mL adiponectin group, the 3 µg/mL adiponectin group, and the non-administered group (P < 0.05) (Figure 8).

3.7. Adiponectin enhances the inhibition of the LN18 cell proliferation rate by TMZ

After treating LN18 cells with 0.1 mM and 1 mM TMZ, the CCK8 assay was employed to assess the proliferation rate. Results indicated that, in comparison with the control group and the vehicle group, TMZ significantly reduced

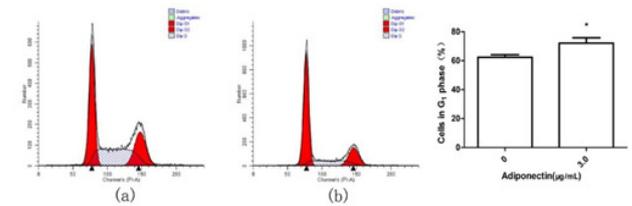


Fig. 7. Effect of adiponectin on LN18 cell proliferation. (a) Control group, (b) Adiponectin 3.0 µg/mL. Compared with the control group, *P<0.05.

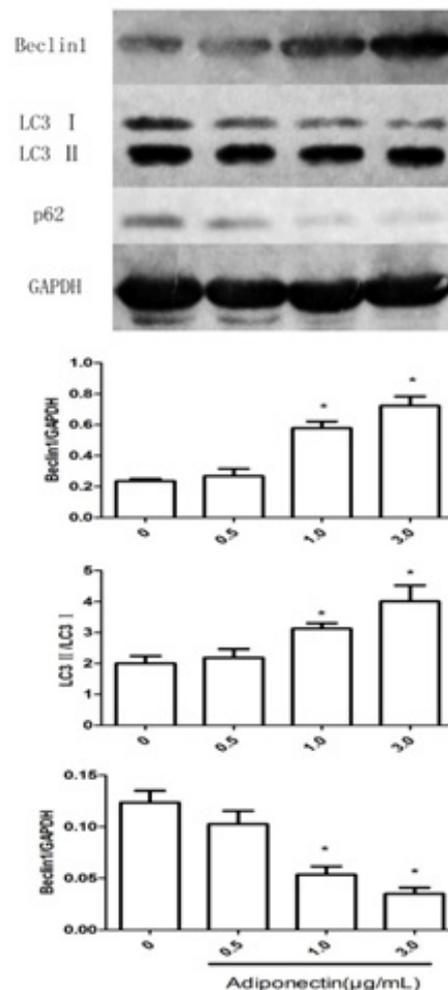


Fig. 8. Effect of adiponectin on the expression of autophagy-related proteins in LN18 cells. Compared with the control group, *P<0.05.

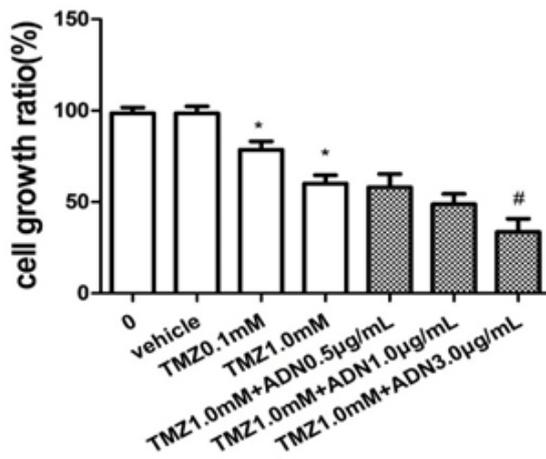


Fig. 9. Adiponectin enhances the inhibitory effect of TMZ on the proliferation rate of the LN18 cell line. Compared with the control group and excipient group, * $P < 0.05$; compared with TMZ 1.0 mM group, # $P < 0.05$.

the cell proliferation rate ($P < 0.05$). Furthermore, when combined with 3 $\mu\text{g}/\text{mL}$ adiponectin, TMZ exhibited an enhanced inhibition of the cell proliferation rate. Notably, the 3 $\mu\text{g}/\text{mL}$ adiponectin+1mM TMZ group demonstrated a significantly lower proliferation rate compared to the 1 mM TMZ group ($P < 0.05$) (Figure 9).

4. Discussion

4.1. Adiponectin and glioma

Adiponectin has been extensively studied for its regulation of tumor cell proliferation and apoptosis, limited research has been conducted on the relationship between adiponectin and glioma. Porcile [5] investigated the effects of adiponectin on glioma cell lines U251 and U87MG. They found that adiponectin activated both the Akt and ERK pathways in glioma cells. However, the activation of the ERK pathway inhibited the proliferation cycle of glioma cells and exerted an inhibitory effect on cell growth. The authors also observed that adiponectin had no effect on the AMPK signaling pathway in glioma cells, and the activation of ERK and Akt was limited and not sustainable [5]. The conclusion of this study differs from our research results, the reasons for this difference are as follows: 1. Their working concentrations are 25 ng/ml and 250 ng/ml , which are 4-40 times lower than the lowest working concentration of 1 $\mu\text{g}/\text{ml}$ obtained through our experiments. Moreover, the normal range of human serum adiponectin is between 2-20 $\mu\text{g}/\text{L}$ [21,22]. The working concentration of adiponectin in our study appears to be within the normal range of human serum adiponectin. The low concentration of adiponectin may be the reason for the lack of AMPK activation, as well as the non-persistent activation of ERK and Akt; 2. The study did not explain the effect of Akt signaling pathway activation on cell apoptosis and proliferation, which may influence the final outcome of the cells. Therefore, we believe that the difference in adiponectin working conditions is the main reason for the inconsistent results between our two studies. When the concentration of adiponectin is between 1 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$, AMPK is significantly activated in PTEN wild-type glioma cells after 24-48 h, exerting its anti-tumor effect. However, in PTEN mutant cells, both AMPK

and Akt are activated, ultimately promoting tumor cell survival. A study by Liu [23] in 2017 indirectly supported our research results. The researchers used U251 and U87MG glioma cells and discovered that miR-3908 can impede the growth of glioma cells by suppressing AdipoR1. When miR-3908 was suppressed, AdipoR1 was activated, leading to decreased apoptosis and increased cell proliferation, which may be linked to AMPK activation. However, the study did not provide a clear explanation for the opposing effects of AMPK activation, despite its downstream mTOR inhibition, which should have anti-apoptotic and anti-proliferative effects on tumors.

In previous investigations, the efficacy of resveratrol in diminishing PTEN-mutant glioma cell populations within U251 has been demonstrated, contrasting with its limited impact on PTEN-wildtype LN18 cells [24]. Lv's exploration revealed that overexpression of DEC1 hampers TMZ-induced apoptosis in LN18 glioma cells, whereas DEC1 knockout accentuates TMZ-induced apoptosis, with these effects not manifesting in U251 cells [25]. Barazzuol documented disparate responses of U251 and LN18 cells to dihydroxyphenylalanine [26]. Lee's observations disclosed distinct survival rates between PTEN-mutant and PTEN-wildtype glioma cells following exposure to ionizing radiation [27]. Additionally, Park's findings indicated augmented invasiveness in response to ionizing radiation specifically in PTEN-mutant glioma cells U87MG and U251, with no such effect observed in PTEN-wildtype LN18 cells [28]. Hence, the expression profile of PTEN in glioma cells emerges as a pivotal determinant influencing the cellular fate of gliomas to a considerable extent.

This experimental segment is dedicated to PTEN-wildtype glioma cells, namely LN18, U251, and U87MG, characterized by the absence of mutations or functional loss in the PTEN gene. PTEN primarily functions to inhibit type I PI3K, a versatile molecule crucial in various essential cellular processes, including cell proliferation and migration. The activation of the type I PI3K pathway plays a pivotal role in the conversion of PIP2 into PIP3, concurrently initiating the oncogenic activity of the Akt signaling pathway [29]. Following the formation of PIP3 by type I PI3K, Akt is recruited to the cell membrane and phosphorylated at Thr308 and Ser473, fostering proliferation while inhibiting apoptosis [29,30]. Sustained Akt activation is indicative of a high malignant degree and an unfavorable prognosis in glioma patients. According to the research, patients exhibiting Akt overexpression display a 1-year disease-free survival (DFS) rate of 59% and a 1-year overall survival (OS) rate of 78%. In contrast, patients without Akt overexpression exhibit a 1-year DFS rate of 91% and a 1-year OS rate of 100% [31,32].

The absence of PTEN may be the primary factor contributing to adiponectin's capacity to activate the Akt signaling pathway in U251 and U87MG cells. Adiponectin demonstrated activation of the AMPK pathway in PTEN-wildtype LN18 cells, with no significant activation of the Akt signaling pathway, ultimately leading to the inhibition of p-mTOR expression. Consequently, adiponectin exhibited distinct regulatory effects on apoptosis and proliferation in LN18 cells compared to U251 and U87MG. Elevated adiponectin concentrations suppressed the proliferation rate of LN18 cells, induced apoptosis, and prompted cell cycle arrest at the G1 phase. These outcomes suggest that adiponectin exerts an anti-tumor effect on PTEN-wildtype

glioma cells, in contrast to PTEN-mutant glioma cells. Therefore, it is posited that the impact of adiponectin on different glioma cell lines is variable. Moreover, the deletion of the PTEN gene influences adiponectin's regulation of glioma cell proliferation and apoptosis to some extent. These findings align with prior research results [26,27,33].

Activation of the AMPK signaling pathway in glioma cells induces G1 phase arrest and apoptosis in LN18 cells by acting on downstream mTOR substrates. Additionally, it can trigger autophagy in LN18 cells. Adiponectin specifically increases the expression of Beclin1 and the ratio of LC3II/I in LN18 cells while decreasing the expression of p62. However, the upregulation of autophagy levels in tumor cells, particularly when subjected to TMZ cytotoxicity, can protect the cells (Discussion in Part 4). This is in contrast to the anti-tumor activity of adiponectin on PTEN-wildtype glioma LN18 in terms of apoptosis and proliferation. Similar conclusions were drawn by Jie [34] after treating glioma cells with AdipoR2. Furthermore, upon comparing the difference in cell proliferation rates between the group treated with TMZ alone and the group treated with both TMZ and adiponectin, it was observed that adiponectin enhances the cytotoxic killing effect of TMZ on glioma cells. This finding suggests that adiponectin can improve the chemotherapeutic sensitivity of PTEN-wildtype glioma cells to TMZ.

The study suggests that while autophagy is significant in the survival and death of tumor cells, its determining factor remains the cell's performance in terms of apoptosis and proliferation. This section of our study explains Liu's research [35]. In U251 glioma cells, the upregulation of the AMPK signaling pathway should promote apoptosis and inhibit proliferation after adiponectin receptor activation. However, the ultimate reason for adiponectin's cytoprotective effect is the activation of Akt. This finding further illustrates the adverse effects of PTEN mutations in glioma on prognosis, such as treatment response and recurrence rate.

4.2. Autophagy and Apoptosis

In LN18 cells, adiponectin upregulated both autophagy flux and apoptosis levels, ultimately inhibiting the growth of LN18 glioma cells. It is speculated that the apoptotic effect may be stronger than the protective effect of autophagy. Autophagy helps maintain energy homeostasis by digesting specific substrates within the cell to generate active molecules. Sometimes, when apoptosis occurs, autophagy flux is enhanced, resulting in different morphological features compared to conventional apoptosis. Cells displaying autophagosome-like morphology are observed during cell death. This may represent a novel cell death mechanism related to autophagy, or it may simply be a concomitant phenomenon preceding cell death. The relationship between autophagy and apoptosis poses an intriguing question.

Autophagy and apoptosis are opposing processes. When cells are damaged or under stress, autophagy is upregulated, allowing cells to digest harmful substrates and provide energy for repairing damage. However, if cells continue to be damaged or are severely damaged, apoptosis is activated, leading to programmed cell death. Currently, certain signaling molecules that encourage apoptosis can hinder autophagy by damaging the structure of autophagy-related proteins. This demonstrates the contrast between auto-

phagy and apoptosis. Liu et al.'s research [36] discovered that triptolide increased autophagy flux while promoting cell apoptosis in U251, U87-MG, and C6 cells. However, the anti-tumor activity of triptolide was related to the activation of JNK and the inhibition of Akt, as protective autophagy and apoptosis mutually inhibited each other. This conclusion is consistent with our research results. Additionally, important signaling molecules such as P53 promote autophagy in the nucleus and inhibit autophagy while promoting apoptosis in the cytoplasm [37]. Autophagy and apoptosis maintain a dynamic balance in cells through mutual restraint [38]. This suggests a competition between apoptosis and autophagy.

Autophagy and apoptosis are believed to promote each other. It is currently understood that the digestion of specific substrates within the cell is significant for cell self-repair and avoiding death when cells are damaged. If the damage persists or exceeds a certain intensity, autophagy flux may have exceeded the cell's limit, and the occurrence of apoptosis may be irreversible [39]. However, autophagy can also degrade and reuse intracellular dysfunctional molecules and damaged organelles, providing energy for the occurrence of apoptosis and creating favorable conditions for it [39].

Furthermore, several studies have confirmed that various signaling molecules participate in cell apoptosis and induce autophagy. DAPK overexpression in primary cultured fibroblasts can induce apoptosis. However, in tumor cell lines such as HeLa and MCF-7, the increase in DAPK expression is accompanied by an upregulation of autophagy levels [40]. Deleting DAPK inhibits the accumulation of caspase-3 and LC-3, leading to the blockade of both apoptosis and autophagy [40], this suggests that DAPK plays a crucial role in both apoptosis and autophagy [41]. Atg5 is a key molecule that initiates autophagy, Yousefi [42] discovered that following neutrophil activation, Atg5 is cleaved into tAtg5, this translocates to the mitochondrial inner membrane, resulting in the release of a significant amount of cytochrome C and the activation of apoptosis. Additionally, Atg5 can interact with FADD to participate in cell apoptosis [43]; In specific conditions, certain protein fragments (Atg6 fragments) or protein complexes (Atg3-Atg12) produced during autophagy can decrease the threshold for activating apoptosis via mitochondrial-dependent pathways [44]. Thus, the connection between apoptosis and autophagy is mutually coordinated and interdependent.

Existing research suggests that autophagy plays a crucial role in maintaining cell survival. In cases where cell death is unavoidable, autophagy can provide energy support for the process. The various modes of cell death, such as apoptosis, necrosis, and autophagy, are linked to cell-specific phenotypes, as well as the nature, intensity, and duration of the stimulating factors. However, it is unclear whether autophagy flux can directly induce cell death. In this study, we found that lipin-1 activated AMPK without activating Akt in the LN18 cell line due to the presence of PTEN. This resulted in the simultaneous activation of apoptosis and autophagy, which may be attributed to the loss of Akt restriction and ultimately led to a stronger apoptotic effect. These findings suggest that lipin-1 exhibits anti-tumor activity in glioma, and that PTEN plays a role in determining its impact to some extent. Understanding the pathological mechanisms of glioma and impro-

ving chemotherapy targeting Lipin-1 are significant.

5. Limitations and Future Directions

By comparing the differential effects of lipin-1 on PTEN wild-type glioma cell lines and PTEN mutant glioma cell lines in terms of cell proliferation, apoptosis, and chemotherapy sensitivity, it is proposed that PTEN may be a crucial regulatory node determining the opposite effects of lipin-1 on different glioma cell lines. However, it cannot be excluded that other aberrant gene expressions within the cells may interfere. In future studies, transgenic approaches could be used to introduce normal PTEN genes into PTEN mutant glioma cell lines. Alternatively, siRNA could be employed to knock out PTEN genes in wild-type glioma cell lines. This would allow for comparative investigations to further elucidate the impact of PTEN on the regulation of tumor proliferation and apoptosis by Lipin-1.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Peng Sun, Kang Huo: Conceptualization, methodology, writing original draft preparation. Fude Liu, Yawen Cheng, Chen Chen: Investigation, software, statistical analysis. Jianfeng Han, Jia Yu: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

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