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CD147 protein expression and Temozolomide resistance in glioma cells: an *Ex vivo* and *In vivo* study

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ABSTRACT

It has been noted that temozolomide resistance occurs in a number of malignancies, including glioma, although the underlying cause of this is unknown. The goal of the study in vivo investigation to show that increased CD147 expression in glioma cells is a factor in their resistance to the chemotherapy drug temozolomide. Proliferation assays, TUNEL assays, reactive oxygen species assays, protein degradation assays, immunohistochemistry, Western blotting, quantitative polymerase chain reactions, and tumorigenicity assays were all carried out. Using the human protein atlas databases, the expression levels of CD147 in different kinds of malignancies were examined. For immunohistochemistry, a total of 7, 12, 19, 15, and 16 glioma samples were taken from para-carcinoma tissue, representing stage I, stage II, stage III, and stage IV gliomas, respectively. The expression of CD147 proteins is correlated with the tumor's aggressiveness. Cell development was slowed by suppressing the expression of the CD147 protein. The expression of the CD147 protein contributed to the emergence of temozolomide resistance. Expression of the CD147 protein reduced mRNA expression. The growth-inhibitory impact of temozolomide on glioma cells was enhanced by the suppression of CD147 protein. Nuclear factor E2-related factor 2 expression and CD147 protein expression showed a significant reciprocal connection with each other (p 0.0001, r2 = 0.3254). In glioma, resistance to temozolomide is due to overexpression of CD147 protein and induction of nuclear factor E2-related factor 2.

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Introduction

A major threat to human health, glioma is distinguished by an unfavorable prognosis and a low survival rate (1). Temozolomide is often the chemotherapy of choice for treating gliomas, however, drug resistance may occur (2). As a result, it's critical to comprehend the process behind temozolomide resistance.

Numerous malignancies, including glioma, have high levels of nuclear factor E2-related factor 2, particularly in glioma stem cells (3, 4). One of the transcription factors that mediate the cellular defence response is nuclear factor E2-related factor 2(5). Genes with an anti-oxidative response element transcriptionally activated by nuclear factor E2-related factor 2, which is implicated in numerous biological processes, including tumour growth, drug resistance, and the activity of cancer stem cells (3, 6, 7). Nuclear factor E2-related factor 2 may be impacted by a variety of post-translational changes. For instance, Kelch-like ECH-associated protein 1 destroys nuclear factor E2-related factor 2 by establishing a dimer complex with it (8).

A transmembrane glycoprotein, CD147 has 269 amino acids (5) and interacts with a wide variety of proteins, including integrins, matrix metallopeptidase, and monocarboxylate transporter. These proteins have been linked to the growth and spread of tumors (9). However, other than cell line research, the response of CD147 to glioma cells and temozolomide resistance is not assessed elsewhere (5). The objective of l of the cell line and in vivo investigation was to show that increased CD147 expression in glioma cells leads to their resistance to temozolomide.

Materials and Methods

Ethical approval

The project has received approval from the institute's animal ethics committee (approval number: 551012CFU). The research adheres to China's regulations on animal experimentation.

Western blotting and immunoprecipitation

Human glioma cell lines U251 and T98G were collected. The Chinese Academy of Sciences in Shanghai, China, Cell Bank of the Type Culture Collection were further extracted and lysed. The lysate was centrifuged, after which it was overnight at 4°C and treated with an anti-TrCP antibody (#4394, Cell Signaling Technology, Tokyo,

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Japan). The above-incubated lysate was combined with 10 l of Protein A/GPlus-Agarose for each immunoprecipitation sample (sc-2003, Santa Cruz Biotechnology, CA, USA). Following three cold immunoprecipitation buffer washes, a complex was added to with the same quantity of buffer. A Western blot analysis was performed on this finished combination.

Polymerase chain reaction in quantitative form

Total RNA was isolated, and PrimeScriptTM RT Master Mix (RR036A, Takara Bio Inc., Shiga, Japan) was used to create cDNA using Green® Fast qPCR Mix (RR430A, Takara Bio Inc., Shiga, Japan) (10).

Immunohistochemistry

The immunohistochemical staining and analysis were done in accordance with the manufacturer's protocol (11).

Test for proliferation

24-well plates with a full medium were used to seed the cells. Using an assay kit (5-ethynyl-20-deoxyuridine, Ribobio, Guangzhou, China), cell proliferation was measured in accordance with the manufacturer's procedure. In order to fix, permeabilize, and dye the cells, 50 mM 5-ethynyl-20-deoxyuridine was treated with them for 6 hours. 1 mg/mL of 4′,6-Diamidine-2′-phenylindole dihydrochloride (Merck KGaA, Darmstadt, Germany) was used to stain the cell nuclei for 20 minutes. Fluorescence microscopy was used to assess the amount of 5-ethynyl-20-deoxyuridine that was incorporated into the cells.

TUNEL test

According to the instructions provided by the manufacturer, the One Step TUNEL Apoptosis Test Kit (C1086, Beyotime Biotechnology, Beijing, China) was used to conduct the TUNEL assay.

Assay for reactive oxygen species

Reactive oxygen species concentrations in the cells were measured using flow cytometry and the reactive oxygen species assay kit (S0033, Beyotime Biotechnology, Beijing, China) (12).

Assay for protein degradation

10 g/mL CHX (#2112, Cell Signaling Technology, Beverly, MA, USA) was incubated with the cells for the allotted amount of time. Following cell collection, Western blotting was carried out in accordance with the manufacturer's instructions.

Tumorigenicity Assay

The male mice were 8 weeks old when 1× 106 cells were put into them in 150 mL of phosphate buffer. Then, from days 1 to 5, intraperitoneal injections of Dimethyl sulfoxide or Temozolomide (Merck KGaA, Darmstadt, Germany) 50 mg/kg were given once daily. Every week, tumour volumes were assessed using a calliper and Eq. 1 (5):

$$\text{Volume} = \frac{\pi (d^2 \times D)}{6} \quad [1]$$

Were, d: the minor axis of the tumor, D: the major axis of the tumor.

Statistical analysis

For statistical analysis, InStat 3.01 from GraphPad Software, San Diego, California, USA, was utilized. One-way analysis of variance (ANOVA) was used for continuous data. The relationship between CD147 and nuclear factor E2-related factor 2 expressions was established using linear regression and Pearson's correlation (5). For post hoc analysis, the Dunnett multiple comparisons test was used, with critical value (q) >2.549 being regarded as significant. If the p-value was less than 0.05, then all findings were deemed significant. The Cancer Genome Atlas Program data were used to determine survival rates and the likelihood that certain variables would co-occur in the data set.

Results

Association of expression of CD147 in glioma tissues and tumor malignancy

Human Protein Atlas datasets were used to investigate the expression levels of CD147 in distinct cancer types. In many malignant tissues, there is an elevated expression of the CD147 protein. A total of 69 patients' glioma and normal brain tissues had various levels of CD147 expression. The expression of CD147 proteins is related to the tumor's aggressiveness (Fig. 1). Due of the high quantity of CD147protein, the CD147promoter is hypomethylated (Fig. 2). Reduced survival was associated with high CD147 protein expression, and this association was especially significant in individuals with stage IV and grade III malignancies.

A p-value is < 0.0001, q-value between normal brain cells vs. para-carcinoma tissue, normal brain cells vs. stage I glioma, normal brain cells vs. stage II glioma, normal brain cells vs. stage III glioma, and normal brain cells vs. stage IV glioma were 2.273, 0.7169, 2.323, 3.529, and

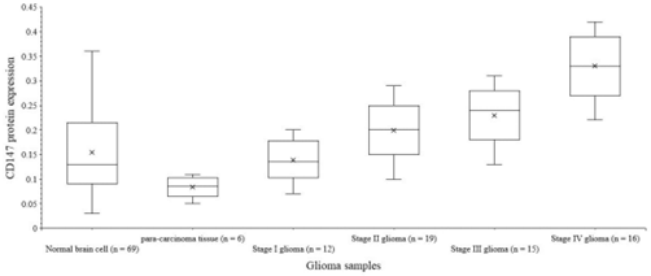

Figure 1. The expression levels of CD147 proteins and different malignancies of the tumor.

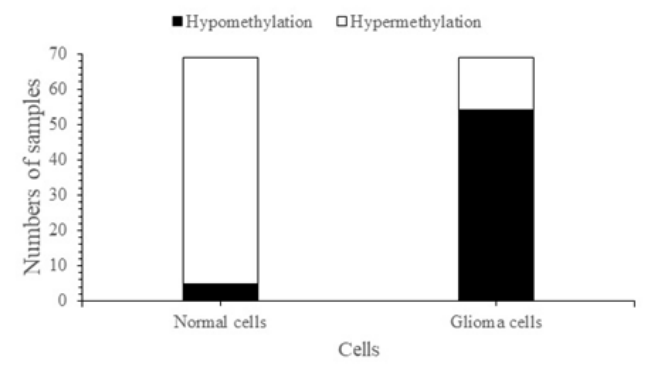

Figure 2. Methylation of CD147 promoter.

8.304, respectively.

According to a proliferation experiment, shRNA-mediated inhibition of CD147 protein expression in glioma cells reduced glioma cell growth. In both the human glioma cell lines U251 and T98G, the expression of the CD147 protein enhanced the inhibitory impact of temozolomide on the survival of cells (Fig. 3). The expression of CD147 protein contributes to the emergence of temozolomide resistance (Fig. 4).

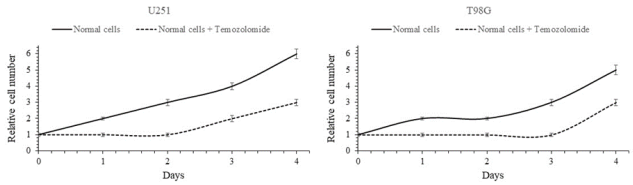

Figure 3. Contributions of CD147 proteins to the resistance to temozolomide treatment.

Quantitative polymerase chain reaction

CD147 protein expression decreased mRNA expressions (Fig. 5). Also, mRNA expressions were decreased in a dose-dependent manner by CD147 protein.

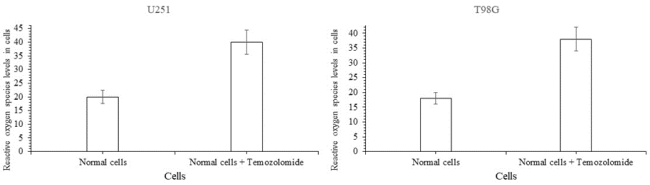

Figure 4. Results of reactive oxygen species assay.

Tumorigenicity assay

Suppression of CD147 protein promoted the inhibitory effect of temozolomide on glioma cell growth (Fig. 6). Nuclear factor E2-related factor 2 increased tumor volume (Fig. 7).

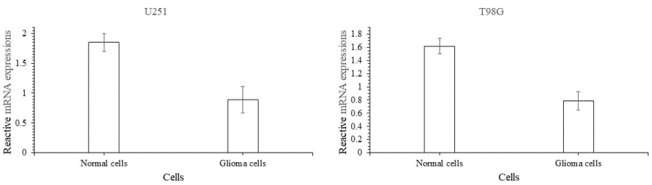

Figure 5. Quantitative polymerase chain reaction.

Correlation between CD147 protein expression and nuclear factor E2-related factor 2 expressions

There was significance reciprocal relationship between CD147 protein expression and nuclear factor E2-related factor 2 expressions ($p < 0.0001$, $r^2 = 0.3254$).

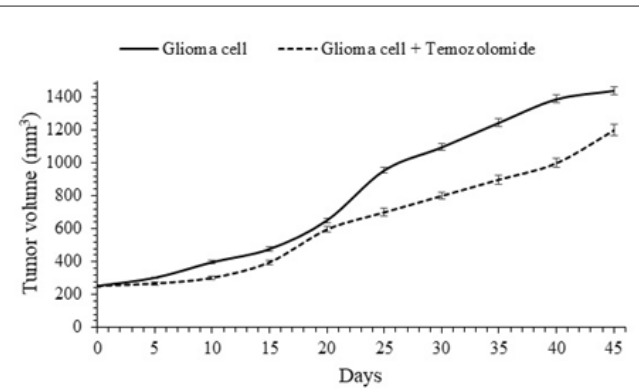

Figure 6. Anti-tumor effect of temozolomide.

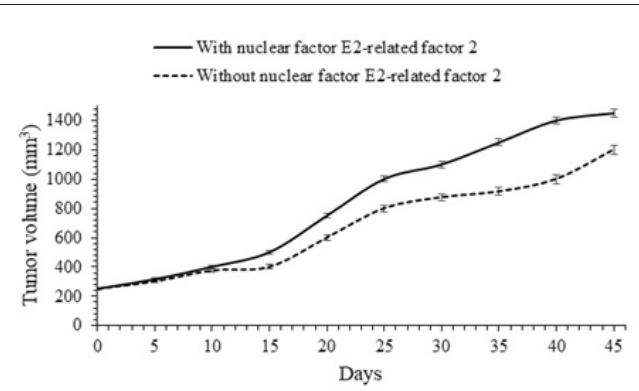

Figure 7. Tumorigenicity assay.

Discussion

Temozolomide is an agent that alkylates DNA. As a result, it is often utilised as the initial chemotherapeutic drug of choice in glioma cases (5). Contrarily, the effect of temozolomide is restricted because glioma cells acquire resistance to it, and the precise causes of this are unknown (13). The development of temozolomide resistance in glioma cells is reportedly linked to redox balance, including the formation of reactive oxygen species (14), according to the available data. In the present research, the generation of reactive oxygen species caused temozolomide resistance, which was counteracted by nuclear factor E2-related factor 2, which was generated by glioma CD147 protein expression. The research's findings agreed with those of a cell line study (5). Nuclear factor E2-related factor 2 is more likely to bind with E3-ligase -TrCP when it is phosphorylated at Ser344 and Ser347 by GSK3 (15, 16). This leads to enhanced ubiquitylation and subsequent proteasomal degradation of nuclear factor E2-related factor 2. According to the research, CD147 protein encourages the stability of nuclear factor E2-related factor 2 protein by inhibiting GSK3-/-TrCP-dependent protein degradation via activation of the Akt pathway.

One of the oncoproteins, CD147, is significantly expressed in a variety of cancer types (5).

Additionally, it performs the role of chemotherapy resistance, albeit the related mechanism of action is unknown. Through the creation of a complex with monocarboxylate transporter 1 in the cell membrane, overexpression of the CD147 protein in multiple myeloma cells leads to the development of immunomodulatory treatment resistance (17). Additionally, the CD147 protein damages DNA causes gemcitabine resistance by focusing on ATM/ATR/p53, and influences the prognosis of pancreatic cancer (18). Although the CD147 protein has been linked to redox hemostasis in several studies, the underlying mechanisms are still unknown (19, 20). The present research discovered that the overexpression of the CD147 protein in glioma tissues is significantly linked with tumour grade (s). Overexpression of the CD147 protein causes glioma cells to become resistant to temozolomide and is associated with

poor results. The research also discovered that CD147 protein inhibited the generation of reactive oxygen species brought on by temozolomide, which caused nuclear factor E2-related factor 2 to be activated. This research might add to our knowledge of the mechanism behind CD147 protein-induced temozolomide resistance in glioma.

The research discovered that overexpression of the CD147 protein induces nuclear factor E2-related factor 2, which is in charge of controlling the formation of reactive oxygen species, redox homeostasis, and temozolomide resistance in gliomas.

Nuclear transcription factor nuclear factor E2-related factor 2 keeps downstream genes expressed.

Additionally, it promotes tumour cell viability (6, 7) and induces tumour metabolic reprogramming (21, 22) to support the development of malignancies.

Nuclear factor E2-related factor 2 induction causes CD147 protein overexpression to be linked to temozolomide resistance in gliomas.

In glioma, resistance to temozolomide is due to overexpression of CD147 protein and induction of nuclear factor E2-related factor 2. This study may provide new evidence for the understanding of the mechanism for CD147 protein-induced temozolomide resistance in glioma.

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Statements and Declarations
The author declares that no conflict of interest is associated with this study.

Authors' contribution
This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

Conflicts of interest
There are no conflicts of interest.

Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

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