A novel STAT3 inhibitor, HJC0152, exerts potent antitumor activity in glioblastoma

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Received February 17, 2019; Accepted March 21, 2019; Epub April 1, 2019; Published April 15, 2019

Abstract: Aberrant expression and activation of signal transducer and activator of transcription 3 (STAT3) is implicated in several malignancies, including glioblastoma, and is correlated with poor outcomes in patients with glioblastoma, rendering STAT3 a potential therapeutic target. However, few STAT3 inhibitors have been approved for clinical use. We recently developed an orally active small-molecule compound with anti-STAT3 activity, HJC0152. This study aimed to test the effect of this novel drug on glioblastoma cell lines, and provide possibility to improve clinic prognosis of patients with glioblastoma in the future. In the present study, we aimed to determine the effects of HJC0152 on the growth, proliferation, and chemosensitivity of glioblastoma cell lines and xenograft tumors. We found that HJC0152 inactivated STAT3 via inhibiting phosphorylation of the Tyr705 residue. In vitro, HJC0152 suppressed the proliferation and motility of glioblastoma cells, induced apoptosis, and enhanced the chemosensitivity of glioblastoma cells. Furthermore, HJC0152 inhibited the growth of glioblastoma xenograft tumors in vivo. This study provides a rationale for developing HJC0152 as a STAT3-targeting therapy for treating human glioblastoma in the future.

Keywords: HJC0152, STAT3, anti-tumor activity, glioblastoma, epithelial-mesenchymal transition, senescence, apoptosis

Introduction

Glioblastoma is the most common and aggressive form of primary malignant brain tumor. Glioblastomas are characterized by their rich intratumor vasculature, infiltrative growth, and resistance to standard therapies including surgery, radiotherapy, and concurrent chemotherapy with temozolomide [1-3]. Even with comprehensive treatment, the median survival time for patients with glioblastoma is only about 15 months, and fewer than 5% of patients with glioblastoma survive for 5 years [4, 5]. Therefore, new molecular targets and novel therapeutic strategies aimed at these targets are urgently needed to improve the prognosis of patients with glioblastoma.

The Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathway is well known for its impact on cell growth, cell differentiation, cell proliferation, tumorigenesis, and cancer progression in both physiological and pathological conditions [6]. When activated by upstream signals, tyrosines on intracellular JAK kinases are phosphorylated, triggering recruitment and phosphorylation of STAT3. Phosphorylated STAT3 then undergoes dimerization, nuclear translocation, and DNA binding and induces transcription of a series of downstream target genes. In many cancers, including lung cancer, gastric cancer, colon cancer, and breast cancer, STAT3 is highly activated and is associated with poor prognosis [7-10]. In glioblastoma, constitutively active STAT3 is involved in cellular chemosensitivity
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[11], proliferation, and apoptosis [12] and is associated with poor prognosis [13, 14], suggesting its potential value as a therapeutic target. Numerous STAT3 inhibitors have been developed and tested in several cancer types in recent years, but none of them has advanced to approval by the Food and Drug Administration (FDA), primarily owing to their poor aqueous solubility or only moderate inhibitory effect [15, 16].

We recently developed a series of O-alkylamino-tethered derivatives, including HJC0152, from niclosamide. Niclosamide is an FDA-approved anticestodal drug with an inhibitory effect on STAT3 signaling [17]. HJC0152 has a better pharmacokinetic profile and stronger STAT3-inhibiting activity than does niclosamide [18]. In recent studies of breast cancer and head and neck squamous cell carcinoma cell lines, we demonstrated that HJC0152 treatment suppressed tumor growth and invasiveness in vitro and in vivo [18, 19]. In this study, we conducted a series of experiments to determine the effects of targeting constitutively active STAT3 with HJC0152 on the growth, aggressiveness, proliferation, senescence, apoptosis, and chemosensitivity of glioblastoma cell lines and xenograft tumors.

Materials and methods

Database analysis

We used the online tool GEPIA (gepia.cancer-pku.cn) to analyze The Cancer Genome Atlas (TCGA) database to compare the expression of STAT3 mRNA between glioblastoma tissue and normal brain tissue and to determine the relationship between STAT3 expression and disease-free survival.

Reagents and cells

The structure and properties of HJC0152, which we synthesized, have been described in a previous publication [18]. The human glioblastoma cell lines U87, U251, and LN229 were obtained from ATCC. U87 and U251 cells were maintained in Eagle’s minimum essential medium (Gibco), and LN229 cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco) under humanized conditions (37°C, 5% CO2). All media were supplemented with 10% fetal bovine serum (FBS, Gibco) and penicillin (100 U/mL)/streptomycin (100 μg/mL) (HyClone).

Cell viability assays

Cells (2000 cells/well) were seeded into 96-well plates and then exposed to HJC0152 (0.01, 0.1, 1, 2, 5, 10, 20, 50, or 100 μmol/L) or dimethyl sulfoxide (DMSO) (1 μL/well) for 24 h. Cell viability was measured with MTT assays (5 mg/mL; Sigma). Cells were incubated for 4 h, then the medium was removed and the MTT crystals were dissolved with DMSO. The absorbance at 490 nm was determined by using a microplate reader (Model 680, Bio-Rad Laboratories Ltd.). The half maximal inhibitory concentration (IC50) was calculated using GraphPad Prism 7.0 software.

Western blotting

Cells treated as above were lysed in radioimmunoprecipitation assay buffer (Solarbio) supplemented with protease and phosphatase inhibitors (Roche). The proteins were separated via 8%, 10%, or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Merck Millipore). The membranes were blocked with 5% nonfat milk in Tris-buffered saline with 0.1% Tween 20, washed, and incubated with primary antibodies (1:1000) and horseradish peroxidase-conjugated secondary antibodies (1:2000). The proteins were visualized with enhanced chemiluminescence reagent (Thermo Fisher Scientific). The primary antibodies used in this study are listed in Table 1.

Migration and invasion assays

For in vitro invasion and migration assays, cells (20,000 cells/well for U87 and U251, 50,000 cells/well for LN229) were seeded in serum-free medium into Transwell inserts (Corning) coated with Matrigel (BD Biosciences) or left uncoated. The receiver plates were filled with medium containing 20% FBS. After incubation at 37°C for 16 h, cells that had penetrated through the pores were fixed with 4% paraformaldehyde (Solarbio) and stained with 0.1% crystal violet (Solarbio). The cells were then washed with phosphate-buffered saline (PBS), and viewed under an inverted microscope (DMI6000B, Leica).

Colony-formation assays

Cells (500 cells/well) were seeded in 2 mL of medium with 10% FBS in 6-well plates over-
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Table 1. Primary antibodies used in this study

<table>
<thead>
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<th>Primary antibodies</th>
<th>Vendor</th>
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mitochondria for attachment. After incubation for 14 days in the presence or absence of HJC0152 (1, 2, or 5 μmol/L for U87 and 0.5, 1, or 2 μmol/L for U251 and LN229) at 37°C, cells were washed twice with PBS and stained with 0.1% crystal violet. Colonies with more than 50 cells were counted under an inverted microscope (DMI6000B, Leica).

Flow cytometry

To determine the proportion of apoptotic cells, cells were treated with DMSO or HJC0152 (2 or 5 μmol/L for U87 and 1 or 2 μmol/L for U251 and LN229), and then collected, washed twice with PBS, and double-stained with fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide (PI) (BD Biosciences). The apoptosis rate was measured using flow cytometry (FACS Canto II, BD Biosciences).

Mitochondrial membrane potential assays

A JC-1 probe (Beyotime Biotechnology) was used to detect mitochondrial membrane potential (ΔΨm) depolarization. Cells were cultured in confocal dishes, treated with DMSO or HJC0152 at designated concentrations for 24 h, and then incubated with JC-1 staining solution at 37°C for 20 min. Cells were then washed twice with PBS. When excited with argon-ion 488-nm and 546-nm lasers, mitochondrial JC-1 monomers and aggregates emit green and red fluorescence, respectively. We estimated ΔΨm by comparing the relative brightness of the green and red fluorescence using FV-1000 laser-scanning confocal biological microscopes (Olympus). An increase in the green/red fluorescence intensity ratio was regarded as indicative of mitochondrial depolarization.

Establishment of xenograft model

10 four-week-old female BALB/c-nu mice were obtained from the Institute of Zoology of Concorde Blood Institute (Tianjin, China). Mice were randomly divided into 2 groups (5 mice in each group), and each mouse was injected subcutaneously with 2 × 10⁶ U87 cells. After 1 week, mice were treated with DMSO or HJC0152 (7.5 mg/kg) daily via intratumoral injection. Tumor volume and mouse body weight were measured and recorded every 3 days. The mice were humanely killed after 4 weeks of treatment, and tumors were collected.
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Statistical analysis

All experiments were repeated at least 3 times. Data are shown as mean ± SD. Differences between treatment groups were assessed using 2-tailed Student t-tests. SPSS software (version 17.0) was used for the statistical analyses. Graphs were illustrated by GraphPad Prism 6 (La Jolla, USA), in which *, **, *** and **** indicated P < 0.05, P < 0.01, P < 0.001, P < 0.0001, respectively. A P value < 0.05 was considered statistically significant.

Results

HJC0152 inhibits constitutive STAT3 activation in glioblastoma cells

We mined the TCGA database using GEPIA and found that STAT3 mRNA was highly expressed in glioblastoma tissues and that high levels of STAT3 mRNA in tumor tissue were associated with poor prognosis in patients with glioblastoma (Figure 1A, 1B). MTT assays determined that the IC_{50} values of HJC0152 in U87, U251, and LN229 glioblastoma cells were 5.396 μM, 1.821 μM, and 1.749 μM, respectively (Figure 1C). When cells were treated with HJC0152 at the IC_{50} for 24 h, the phosphorylation level of STAT3 at Tyr705 was markedly reduced in all 3 glioblastoma cell lines, while the expression of...
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total STAT3 and the level of STAT3 phosphorylation at Ser727 was not affected (Figure 1D). In addition, phosphorylation of PI3K and Akt, which are thought to be upstream proteins of STAT3, was affected by HJC0152. As shown in Figure 1D, expression levels of p-PI3K (Tyr458) and p-Akt (Ser473) were reduced in HJC0152-treated cells.

**HJC0152 suppresses migration and invasion in glioblastoma cells**

The effect of HJC0152 on migration and invasion in glioblastoma cell lines was measured with Transwell assays. We found that HJC0152 exposure significantly impaired the migration and invasion of U87, U251, and LN229 cells (P < 0.01) (Figure 2A, 2B). Because studies have shown that epithelial-mesenchymal transition (EMT) is an important mechanism of cell migration and tumor metastasis that is relevant to clinical outcomes [20, 21], we determined the expression of key markers of EMT in glioblastoma cell lines upon treatment with HJC0152. As shown in Figure 2C, expression levels of Twist1, vimentin, N-cadherin, and matrix metalloproteinases (MMP) 2/9 were reduced after treatment with HJC0152, whereas expression of E-cadherin was increased. Taken together, these results suggest that HJC0152 inhibited EMT and suppressed the motility of glioblastoma cells in vitro.
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Colony-formation assays were used to assess glioblastoma cells’ capacity for survival and proliferation in the presence of HJC0152. In these assays, cells treated with HJC0152 formed significantly fewer and smaller colonies than did control cells treated with DMSO. Figure 3. HJC0152 inhibits the growth of glioblastoma cells. (A, B) Representative images (A) and quantification (B) of colony-formation assays of U87, U251, and LN229 cells treated with the indicated concentration of HJC0152 or DMSO (control). (C, D) Flow cytometry results showing cell-cycle distribution of glioblastoma cells treated with HJC0152 or DMSO. (E) Representative Western blots showing expression of Cyclin D1 and p21 in DMSO- or HJC0152-treated cells. Three independent experiments were performed. **P < 0.01; ***P < 0.001; ****P < 0.0001.

HJC0152 inhibits proliferation and induces senescence in glioblastoma cells

Colony-formation assays were used to assess glioblastoma cells’ capacity for survival and proliferation in the presence of HJC0152. In these assays, cells treated with HJC0152 formed significantly fewer and smaller colonies than did control cells treated with DMSO.

HJC0152 inhibited proliferation of all 3 glioblastoma cell lines in a concentration-dependent manner (P < 0.01) (Figure 3A, 3B). We next used flow cytometry to determine the mechanism by which HJC0152 inhibited cell proliferation. As shown in Figure 3C, 3D, the percentage of cells in G1 phase increased after 24 h of HJC0152 treatment in U87, U251, and LN229 cells. Western blotting of cell-cycle markers
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We detected a remarkable decline in the expression of cyclin D1 and an increase in p21 expression upon HJC0152 treatment (Figure 3E).

We next employed SA-β-gal staining to assess the rates of glioblastoma cell senescence induced by HJC0152. Compared to DMSO-treated cells, HJC0152-treated U87, U251, and LN229 cells displayed significantly elevated SA-β-Gal activity (P < 0.001; Figure 4A, 4B), indicating increased cell senescence. Finally, we analyzed changes in p16 expression, which regulates G₁-S transition, upon HJC0152 treatment. We found that p16 levels were higher in HJC0152-treated cells than in DMSO-treated cells (Figure 4C). Taken together, these results indicate that HJC0152 inhibits the proliferation of glioblastoma cells by arresting cells in G₁ phase and inducing cell senescence.

HJC0152 induces apoptosis in glioblastoma cells

We employed FITC-Annexin V/PI staining to compare rates of apoptosis in DMSO- and HJC0152-treated glioblastoma cells. HJC0152

Figure 4. HJC0152 induces senescence of glioblastoma cells. (A, B) Representative photomicrographs (A) and quantification (B) of induction of senescence in glioblastoma cells by HJC0152, assessed by SA-β-gal staining. Magnification × 100 (C) Western blots showing expression level of p16. Three independent experiments were performed. ***P < 0.001; ****P < 0.0001.
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A

DMSO

U87

HJC0152

2μM

10.45%

5μM

21.71%

DMSO

1μM

10.43%

2μM

15.13%

DMSO

1μM

6.922%

2μM

12.38%

B

U87

Apoptosis (%)

DMSO

2μM

5μM

U251

Apoptosis (%)

DMSO

1μM

2μM

LN229

Apoptosis (%)

DMSO

1μM

2μM

C

U87

U251

LN229

DMSO

HJC0152

DMSO

HJC0152

DMSO

HJC0152

pro-caspase3

-35kDa

cleaved caspase3

-19kDa

Bcl-2

-26kDa

Bax

-23kDa

GAPDH

-39kDa
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Figure 5. HJC0152 induces apoptosis of glioblastoma cells. A, B. Annexin V/propidium iodide (PI) staining showing rates of apoptosis after treatment with the indicated concentrations of HJC0152 or DMSO (control). C. Western blots showing expression levels of apoptosis-associated proteins. Three independent experiments were performed. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Figure 6. HJC0152 induces loss of mitochondrial membrane polarization and translocation of Bax and cytochrome C in glioblastoma cells. A. A JC-1 probe was used to detect changes in mitochondrial membrane polarization in U87, U251, and LN229 cell lines. Magnification × 200. B. Western blots showing protein translocation in mitochondria extracted from glioblastoma cells treated with HJC0152 or DMSO (control). Three independent experiments were performed. CYTO, cytoplasm; MITO, mitochondria; Cyto C, cytochrome C.

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Figure 6. HJC0152 induces loss of mitochondrial membrane polarization and translocation of Bax and cytochrome C in glioblastoma cells. A. A JC-1 probe was used to detect changes in mitochondrial membrane polarization in U87, U251, and LN229 cell lines. Magnification × 200. B. Western blots showing protein translocation in mitochondria extracted from glioblastoma cells treated with HJC0152 or DMSO (control). Three independent experiments were performed. CYTO, cytoplasm; MITO, mitochondria; Cyto C, cytochrome C.

treatment induced apoptosis in U87, U251, and LN229 cells in a concentration dependent-manner (P < 0.05) (Figure 5A, 5B). Furthermore, Western blotting of apoptosis-associated proteins showed lower expression of B-cell lymphoma 2-family protein (Bcl-2) and elevated Bcl-2-associated X protein (Bax) in HJC0152-treated cells. In addition, loss of pro-caspase 3 and gain of cleaved caspase 3 expression were observed after HJC0152 treatment (Figure 5C).

The loss of ΔΨm is considered to be an early event in apoptosis [22]. We used a JC-1 fluorescent probe to detect ΔΨm to estimate the effect of HJC0152 on mitochondrial apoptosis in glioblastoma cells. As shown in Figure 6A,
exposure to HJC0152 for 24 h led to dramatic reductions of ΔΨm in all 3 cell lines. Because loss of ΔΨm is related to recruitment of Bax to the mitochondria and leads to outflow of cytochrome C to the cytoplasm, we separated the mitochondria from the cytoplasm and measured the abundance of Bax and cytochrome C in the cytoplasm and mitochondria of DMSO- and HJC0152-treated cells. Western blot assays demonstrated that more Bax bound to mitochondria and more cytochrome C escaped to the cytoplasm in HJC0152-treated cells than in DMSO-treated cells (Figure 6B), a phenomenon that characterizes early apoptosis. Collectively, these results suggest that HJC0152 induces mitochondria-associated apoptosis in glioblastoma cells in vitro.

**HJC0152 increases cisplatin’s cytotoxicity in glioblastoma cells**

To assess the effect of HJC0152 and cisplatin used in combination, U87, U251, and LN229 cell lines were treated with cisplatin alone or with a combination of HJC0152 and cisplatin. We then determined the IC_{50} for cisplatin. Cisplatin alone inhibited the growth of glioblastoma cells and addition of HJC0152 further sensitized the cells to cisplatin. As shown in Figure 7A, the IC_{50} values of cisplatin in U87, U251, and LN229 cell lines were 10.37 μM, 10.84 μM, and 22.45 μM, respectively. After preincubation with HJC0152 for 6 h, the IC_{50} of cisplatin in these cell lines dropped to 3.488 µM, 3.885 μM, and 5.966 μM, respectively. Addition of HJC0152 to cisplatin also increased the inhibitory effect of cisplatin on the colony-forming capacity of glioblastoma cells. Figure 7B, 7C show that treatment with cisplatin alone approximately halved the number of colonies formed, while addition of HJC0152 further reduced the number of colonies.

**HJC0152 suppresses glioblastoma tumor growth in vivo**

To evaluate HJC0152 as a potential therapeutic agent for patients with glioblastoma, we established a U87 xenograft tumor model. In this model, HJC0152 demonstrated a potent suppressive effect on tumor growth. Both tumor volume and weight were significantly lower in
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mice that had been treated with HJC0152 than in DMSO-treated mice ($P < 0.05$) (Figure 8A-D). Furthermore, HJC0152 administration did not cause significant loss of body weight in tumor-bearing mice over the duration of the experiment (Figure 8E). These results demonstrate that HJC0152 has strong antitumor effects and minimal toxicity.

Discussion

In this study, we present evidence that HJC0152, a novel STAT3 inhibitor, inhibits the growth and motility of glioblastoma cells, induces senescence and apoptosis of glioblastoma cells in vitro, and suppresses the growth and progression of glioblastoma xenograft tumors in vivo.

HJC0152 is a novel O-alkylamino-tethered derivative from niclosamide, which is a moderate STAT3 inhibitor but has poor solubility in water [18]. HJC0152 has better anticancer activity and approximately 680-fold better aqueous solubility than does niclosamide, making HJC0152 a promising candidate for future clinical translation.

In this study, we verified that HJC0152 inhibits STAT3 activation in glioblastoma cell lines. In particular, HJC0152 inhibits phosphorylation of STAT3 at Tyr705 in the SH2 domain, a hallmark of STAT3 activation that is essential not only for STAT3 dimerization, but also for STAT3-DNA binding [15, 23]. Once inhibited, STAT3 was no longer able to function normally to join in the transcription of its target genes, such as cyclin D1, Bcl-2, survivin, Twist1, vimentin, and MMP2/9 [24-29].

Several of STAT3’s downstream proteins are involved in EMT and cellular motility. We demonstrated that HJC0152 treatment impairs cellular motility and reverses EMT in glioblastoma cells. Twist1 is a basic helix-loop-helix transcription factor involved in EMT and invasion in glioblastoma by upregulating expression of Snail2, HGF, FAP and FN1 [30]. Vimentin, as a member of the intermediate filament family, participates in modulating the motility and invasion of cancer cells and is often used as a marker of EMT [31]. In glioblastoma, vimentin expression is negatively associated with patient prognosis [32]. Cadherin modulation is part of the oncogenic EMT program; in this process, cells gain N-cadherin and lose E-cadherin [33]. MMP2 and MMP9, which are known to play an essen-
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tial role in cancer invasion and metastasis, are often overexpressed in aggressive brain tumors and act as negative prognostic factors [34-37]. Thus, we conclude that HJC0152 disrupts EMT in glioblastoma cells by impairing transcriptional activity of STAT3 and downregulating expression of STAT3 downstream target genes.

Our results also demonstrate that HJC0152 suppresses proliferation and induces senescence of glioblastoma cells, with corresponding alterations in biomarkers of these processes. Cyclin D1 and p21 mediate G₁-S phase cell cycle progression. When cyclin D1 decreases and p21 increases, cells are arrested in G₁ phase [38, 39]; our Western blot and flow cytometry results are consistent with this. Cellular senescence characterized by the loss of proliferation ability in viable, metabolically active cells, even when sufficient nutrients and mitogens are available. Studies have proved that the program of senescence contributed to the outcome of cancer therapy [40]. STAT3 regulates senescence via several mechanisms that depend on the cellular context [41]. For example, in normal human fibroblast TIG3 cells, STAT3 promoted premature senescence via the STAT3-IGFBP5 axis [42]. However, in HCT116 human colon cancer cells and murine breast cancer cells, inactivation of STAT3 caused a senescent phenotype [43, 44]. In addition, interleukin-6, a stimulator of the JAK/STAT3 pathway, was shown to possess antisenescence activity in the tumor microenvironment [45]. In our study, inhibition of STAT3 induced senescence in glioblastoma cells and promoted the expression of p16, a protein controlling the G₁-S transition and a marker of senescence [46].

We also determined the mechanism by which treatment with HJC0152 induces apoptosis in U87, U251, and LN229 glioblastoma cells. In the presence of HJC0152, expression of Bcl-2 was reduced and that of Bax was elevated, and more caspase 3 was cleaved. Moreover, we observed that HJC0152 treatment increased mitochondria-associated apoptosis. Mitochondria are the master regulatory site of cell-death signaling. When cellular homeostasis is disrupted by detrimental environmental signals, mitochondria trigger apoptosis [47]. Bax translocation and cytochrome C release are key upstream molecular events of apoptosis. Bax and other proapoptotic proteins contribute to outer-membrane permeabilization by interacting with voltage-dependent anion channels, leading to the collapse of ΔΨm, release of cytochrome C into the cytosol, and activation of caspases [48-51]. We observed both Bax translocation to mitochondria and cytochrome C release to the cytoplasm following HJC0152 treatment. Furthermore, ΔΨm was significantly depolarized.

We also demonstrated that HJC0152 improves glioblastoma cells' sensitivity to treatment with cisplatin, an effective antitumor drug that has been employed to treat a wide range of solid tumors [52] and is used as an adjuvant therapy in gliomas [53]. Glioblastomas generally respond poorly to chemotherapy, including cisplatin-based regimens [54]. We found that the IC₅₀ of cisplatin was lower in HJC0152-pretreated glioblastoma cells and that the combination of HJC0152 and cisplatin synergistically inhibited cell growth compared with cisplatin treatment alone.

Our glioblastoma xenograft tumor model further confirmed the therapeutic potential of HJC0152. Tumors treated with HJC0152 grew much more slowly than did those treated with DMSO, demonstrating that HJC0152 has potent anticancer activity towards glioblastoma xenograft tumors. During the period of treatment, the body weight of the mice did not vary significantly, suggesting that HJC0152 has an acceptable toxicity profile in vivo. Although we employed intratumoral injection of HJC0152 in these experiments, orally administered HJC0152 inhibited the growth of xenograft tumors in a previous study, demonstrating the bioavailability of this agent [18].

Although HJC0152 pithily experts anti-tumor activity in glioblastoma and has been shown to inhibit the growth of several other types of cancer [18, 19, 55], the molecular mechanisms by which HJC0152 exerts its anticancer efficacy via STAT3 still remain to be explored. Moreover, it is not certain if this drug specifically acts on STAT3, or it harasses other signaling pathways as well. More details of how HJC0152 effects on the organism is worthy of being further studied in the coming future.

In conclusion, the anticancer activity and bioavailability of HJC0152 makes it a promising
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new therapy for patients with glioblastoma. Our study provides a rationale for developing HJC0152 as a novel therapy for patients with invasive, progressive, and metastatic glioblastoma.

Acknowledgements

This work was supported in part by grants from the National Science Foundation of China (NSFC81572492), National Clinical Research Center for Cancer (NCRCC) and Supported by Special Program of Talents Development for Excellent Youth Scholars in Tianjin. We thank Amy Ninetto, PhD, ELS, Department of Scientific Publications, The University of Texas MD Anderson Cancer Center for her editing of the manuscript.

Disclosure of conflict of interest

None.

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