Dendritic Cells Loaded With Heat Shock Inactivated Glioma Stem Cells Enhance Antitumor Response of Mouse Glioma When Combining With CD47 Blockade

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ABSTRACT

BACKGROUND: For glioma patients, the long-term advantages of dendritic cells (DCs) immunization remain unknown. It is extremely important to develop new treatment strategies that enhance the immunotherapy effect of DC-based vaccines. DCs exposed to glioma stem cells (GSCs) are considered promising vaccines against glioma.

METHODS: Glioma stem cells were isolated from mouse glioma GL261 cells (GCs). Both were subjected to severe (47°C) and mild (42°C) heat shock to induce immunogenic cell death (ICD). Membrane mobilization of calreticulin (CRT) and release of heat shock proteins (HSPs) were detected by flow cytometry. Dendritic cells were then exposed to heat-inactivated cells and co-culturing of T cells tested for immunotherapeutic efficacy in vitro. In vivo, we investigated the GSC targeting effect of the GSC-DC vaccine combined with CD47 blockade.

RESULTS: Heat shock induced ICD in GCs and GSCs, as indicated by significant release of calreticulin, HSP70, and HSP90. Heat shock condition ICD lysates induce maturation and activation-associated marker expression on monocyte-derived DCs. Accordingly, DCs pulsed with GCs and GSCs inactivated reduced colony formation, sphere formation, migration, and invasion of glioma and GSCs in vitro. Glioma stem cell-DC vaccine in combination with anti-CD47 antibody significantly enhanced survival in mice with glioma, induced production of interferon (IFN)-γ, and enhanced T-cell expansion in vivo. Of note, DCs pulsed with inactivated GSCs were more effective to control tumor growth than DCs pulsed with inactive GCs.

CONCLUSIONS: Severe heat shock induces ICD in vitro. These data showed that administration of anti-CD47 antibody combined with GSC-DC vaccine may represent an effective immunotherapeutic strategy for cancer patients in clinical.

KEYWORDS: Glioma, glioma stem cell, CD47, immunogenic cell death, combination therapy

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Introduction

Glioma is one of the most common primary tumors in the adult central nervous system[1] and is typically associated with poor prognosis. The standard treatment is surgical resection followed by radiotherapy and adjuvant chemotherapy. Although some progress has been made in the treatment of gliomas with dendritic cell (DC) vaccines, clinical outcomes for glioblastoma remain poor, and new treatments are needed.[2]

Of note, numerous tumors, including glioma, are well-established to contain a small subset of cells with stem cell characteristics.[3] These cells, also known as cancer stem cells, are believed to promote tumor progression and have potential to self-renew, proliferate, differentiate into multiple lineages, and form spheres.[4,5] In addition, these cells are undifferentiated and typically express CD133 and Nestin.[6-9] Standard treatments like chemotherapy do not effectively eradicate these cells,[10,11] which then continue to proliferate and differentiate, ultimately leading to tumor recurrence. Thus, elimination of glioma stem cells (GSCs) may significantly improve patient survival.

Of alternative glioma treatments currently in development, immunotherapy is theoretically very attractive due to its potential for high tumor-specific cytotoxicity.[12-14] However, glioma cells are poor antigen-presenting cells and express antigens that do not appear to be immunogenic in themselves. On the contrary, DCs are excellent antigen-presenting cells that mediate immune surveillance, antigen capture, and antigen presentation.[15,16] Strikingly, DC vaccines loaded with tumor-derived

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antigens elicit antitumor humoral and cellular immunity, and such vaccines have been tested against several cancers.[17] Recently, several reports indicated that DCs exposed to GSCs also induce effective antitumor immunity, and hence may improve clinical outcomes.[18,19] Nevertheless, optimization of antigen loading into DCs remains urgently needed to improve efficacy.

Severe (>43°C), but not mild (<42°C) heat shock was reported to induce immunogenic cell death (ICD) in cancer cells.[20] ICD is primarily achieved by generation of damage-associated molecular patterns (DAMPs) such as active secretion of adenosine triphosphate (ATP) and preapoptotic surface exposure of calreticulin and heat shock proteins (HSP70 and HSP90) from the endoplasmic reticulum.[21] Other DAMPs generated in late-stage apoptosis include extracellular release of high-mobility group box 1 protein (HMGB1).[21] In turn, these patterns engage a series of receptors in DCs to stimulate presentation of tumor antigens to T cells. Whether DCs loaded with antigens from heat-inactivated cells are therapeutic against glioma treatment has not been clearly established.

In recent years, the successful use of immune checkpoint blockage (ICB) has re-energized the area of cancer immunotherapy. While some patients had partial or even complete responses to ICB therapy, many others remained unresponsive.[22] CD47 (integrin-associated protein) is a ubiquitously expressed transmembrane glycoprotein. CD47 acts as an antiphagocytic "don't eat me" signal, binding signal regulatory protein (SIRP)-α on the surfaces of macrophages. Activation of SIRP-α results in the inhibition of phagocytosis. We previously reported that anti-CD47 mAb inhibited tumor growth in an immunocompetent syngeneic glioma model.[23] Clearly, cancer vaccines and ICB have flaws of their own. Cancer vaccines may create tumor-specific T-cells, which could be boosted by ICB to combat immune-suppressive processes. To test this hypothesis, we focused on targeting GSCs using the GSC-DC vaccine in this study while simultaneously blocked CD47 to investigate the effects of the combined glioma immunotherapy.

In this study, GSCs were isolated from mouse glioma GL261 cells. Subsequently, both tumor and isolated stem cells were subjected to severe (47°C) and mild (42°C) heat shock. DCs were then pulsed with such heat-inactivated cells, and tested in vitro. ICD-based DC vaccines, in combination with anti-CD47 antibody were tested in an immunocompetent syngeneic glioma model. These data showed that the combination therapy exhibit excellent antitumor immunity against mouse glioma.

Materials and Methods

Mice and cell line

Male C57BL/6 mice 6 to 8 weeks were purchased from the Animal Experiment Center of Southern Medical University (Guangzhou, China) and housed specific pathogen-free. Animal care and euthanasia were carried out with the approval of the Animal Care and Use Committee of Zhujiang hospital Southern Medical University, on July 24, 2020 (Guangzhou, China) (approval no: LAEC-2020-101). Mice were sacrificed following bioethical guidelines. The mouse glioma cell line GL261 is archived at the Department of Neurosurgery, Zhujiang Hospital, Southern Medical University (Guangzhou, China), and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (Gibco, USA) and in a humidified incubator at 37°C and with 5% CO_2.

Tumor sphere culture and isolation of GSCs

GL261 cells were cultured in serum-free DMEM/F12 containing 20 ng/mL recombinant epidermal growth factor (EGF), 20 ng/mL bFGF, and 2% B27 (Life Technologies, USA). The resulting tumor spheres were cultured in the same medium and passaged under the same conditions for up to 1 month. CD133+ cells were then isolated by fluorescence-activated cell sorting (BD Biosciences).

Isolated cells were then analyzed for the stem cell markers CD133 and Nestin by flow cytometry and immunofluorescence. For flow cytometry, cells were digested with 0.05% trypsin (Gibco, USA) for 5 min, centrifuged at 1000 r/min for 5 min, and resuspended in phosphate-buffered saline (PBS). Cells were then fixed with 4% paraformaldehyde for 15 min at 4°C, treated with 0.1% TritonX-100, and stained with PE-labeled rabbit antibodies to CD133 (1:100, Ebioscience,USA) or Nestin (1:100, Ebioscience,USA). For immunofluorescence, cells were fixed with 4% paraformaldehyde for 30 min at 4°C, permeabilized with 0.3% Triton X-100, and blocked with 5% naïve goat serum. Samples were then probed for 1 h with rabbit anti-CD133 (1:200, Ebioscience, USA) or mouse anti-Nestin (1:100, Abcam, USA), and labeled with goat antirabbit IgG conjugated to Alexa Fluor 594 (1:200, Invitrogen, USA) or with anti-mouse IgG conjugated to DyLight 488. Nuclei were counterstained with DAPI (Invitrogen, USA).

Spheroids were also incubated in DMEM/F12 containing 10% fetal bovine serum (Gibco, USA) for 5 to 10 days. Expression of glial fibrillary acidic protein (GFAP, Abcam, ab10062, USA), SOX2 (Abcam, ab79351, USA), and Galc (Thermo Fisher, PA5-109753, USA) were then analyzed by immunofluorescence to assess differentiation.

Heat-inactivation of GCs and GSCs

Glioma GL261 cells and GSCs were plated at 1×10^6 cells/mL, cultured at 37°C for 24 h, heated for 1 h at 42°C or 47°C, and incubated at 37°C for another 12 h and 24 h. Unheated cells were used as control. After treatment, cells were incubated for 1 h at 4°C with anti-CRT (1:200, Abcam, USA) or anti-HSP90 (1:200, Abcam, USA) or anti-HSP70 (1:200, Santa Cruz, USA) antibodies. Flow cytometry was performed on a BD FACSAria (BD Biosciences). In another case, after treatment, intracellular and secreted ATP was measured in the

conditioned media via ATP Bioluminescent assay kit (1:200, Sigma, Germany).

Isolation of bone marrow-derived DCs

Bone marrow-derived monocytes were cultured for 5 days in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum, 20 ng/mL recombinant GM-CSF (PeproTech, USA), and 20 ng/mL recombinant interleukin (IL)-4 (PeproTech, USA). Media were replaced on day 3 and day 5, and immature DCs were harvested on day 6.

Maturation of DCs

Dendritic cells were mixed 1:3 with heat-inactivated GCs or GSCs, and incubated for 24 h. Dendritic cells stimulated with 100 ng/mL lipopolysaccharide for 24 h were used as positive control. Expression of DC maturation markers were assessed via flow cytometry, using the following monoclonal antibodies (mAbs): Fluorescein isothiocyanate (FITC)-conjugated anti-H-2Kb (1:200, BD, USA), PE-conjugated anti-I-A/I-E and anti-CD40 (1:200, BD, USA), and PE-conjugated anti-CD80 (1:200, BD, USA), and anti-CD86 (1:200, eBioscience, USA) and anti-MHC II (1:200, eBioscience, USA). For each staining the appropriate isotypes were used. Analysis was performed using the Cellquest software on a FACSort cytometer (BD Biosciences). For immunization, matured DCs were washed 3 times with PBS and resuspended in PBS at 1×10^7 cells/mL. Supernatants from matured DCs were also collected and assayed by enzyme-linked immunosorbent assay (ELISA) kits to quantify secretion of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-6, and IL-10, following the manufacturer's instructions. Each sample was tested in triplicate to obtain mean pg/ml and standard deviations.

T-cell culture, purification, stimulation, and cytotoxicity assay

Splenocytes were obtained from healthy C57BL/6 mice, disaggregated by passing through a 40-μm nylon filter and treating with 1 mL of erythrocyte lysis buffer for 3 min, and diluted to 1×10^8 cells/mL. CD3 + T lymphocytes were isolated by magnetic-activated cell sorting, using EasySep™ Mouse T-Cell Enrichment Kit (Stem Cell Technologies, Canada), carefully following product instructions. The purity of isolated T lymphocytes was assessed immediately after sorting by flow cytometry based on FITC-labeled anti-CD3 (1:100, BD bioscience, USA). The ratio of CD3+ T lymphocytes was 95.8%. Finally, isolated T-cells were resuspended in RPMI-1640 supplemented with 100 U/mL recombinant murine IL-2 (PeproTech, USA), cultured, and stimulated at 10:1 with loaded DCs for 3 days. T-cells stimulated with unloaded DCs were used as control. Using a CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega, China), GCs and purified GSCs were seeded into separate 96-well plates as target cells, and co-cultured at 1:20 with stimulated T-cells. Incubate cells at 37°C for the desired test exposure period. Release of lactate dehydrogenase was quantified after 4 h following the manufacturer's instructions. Experiments were independently repeated 3 times.

Colony formation

Glioma GL261 cells were co-cultured with stimulated T lymphocytes and seeded in 6-well plates at 100 cells/well (n = 2 wells per treatment). After 14 days at 37°C, colonies were washed twice with PBS and stained with hematoxylin. Colonies composed of more than 50 cells were counted in each well under a microscope. All experiments were repeated at least 3 times.

Transwell migration and Boyden invasion

Glioma GL261 cells were co-cultured for 24 h at 1:20 with stimulated T-cells, washed 3 times with PBS to remove floating cells, collected, and counted. Cells were then seeded in serum-free DMEM at 1×10^5 cells/well into the upper chamber of a transwell dish (BD Biosciences, MA). The lower chamber was filled with DMEM containing 10% fetal bovine serum. After 12 h, remaining cells in the upper chamber were scraped out by cotton swab, while cells that had migrated to the lower surface of the membrane were fixed with methanol, stained with crystal violet (Sigma), and counted in 5 random optical fields. Boyden invasion was assessed in a similar manner except that a matrigel (BD Biosciences, MA) was added to the upper chamber. Assays were independently performed 3 times.

Sphere formation

Glioma GL261 cells were co-cultured for 24 h with stimulated T-cells at different ratios, collected, counted, plated in ultra low-attachment 6-well plates, and grown in serum-free DMEM/F12 supplemented with 20 ng/mL EGF, 20 ng/mL bFGF, and 1:50 B27 (Gibco, USA). After 1 week, tumor spheres >100 μm were counted under an inverted microscope.

Generation of a mouse brain tumor model

Cells transduced with the lentivirus vector pUbi-MCS-LUC-IRESpuromycin (GENE) were used for the orthotopic model. After anesthesia (induced using ketamine and xylazine hydrochloride), mice were placed in a stereotactic frame. A burr hole was drilled through the skull and glioma cells injected with a 26-gauge syringe (Hamilton) at a position 1-mm lateral and 2-mm posterior of the bregma and to a depth 3 mm below the dura mater. After a week, tumor formation was verified using bioluminescence imaging (BLI). After intracranial inoculation,

mice were continuously monitored and clinical symptoms were scored with a neurological deficit scale adapted from our previously described reports 3 times a week, with grade 0 for healthy mice, grade 1 for slight unilateral paralysis, grade 2 for moderate unilateral paralysis and/or beginning hunchback, grade 3 for severe unilateral or bilateral paralysis and pronounced hunchback, and grade 4 for moribund mice.[23]

Cytokine detection by ELISA

Immature DCs derived from the bone marrow progenitor cells of C57BL/6 mice were loaded with heat shock-inactivated GC and GSC lysates. Then supernatants were collected and centrifuged at 15 000 r/min at 4°C for 3 minutes. The supernatants were used immediately for IL-6, IL-10, TNF-α, and IFN-γ measurements respectivly by ELISA kit (PeproTech, Germany). The absorbance was measured at 450 nm with a microplate reader.

After 2 weeks of the treatment beginning, the peripheral blood of mice in each group was collected and centrifuged at 3000 r/min at 4°C for 20 minutes. The cytokine levels of IFN-γ collected from the serum was detected by ELISA kit (PeproTech, Germany) according to the manufacturer's instructions. The absorbance was measured at 450 nm with a microplate reader. The experiments were conducted independently 3 times.

Statistical analysis

Data are reported as mean ± standard deviation, and were analyzed in SPSS 20.0 and Graphpad 7.0. One-way analysis of variance was used to compare multiple groups. P values $<.05$ were considered statistically significant and are marked * for $P<.05$, ** for $P<.01$, and *** for $P<.001$.

Results

GL261 tumor spheres have properties of GSCs

To test whether GSCs can be obtained, GL261 cells were cultured in bFGF, EGF, and B27 without serum. After 5 days, free-floating tumor spheres were observed. These spheres can be dissociated mechanically and can be passaged for at least 1 month (Figure 1A). The spheres were then assayed by immunocytochemistry and flow cytometry for CD133 and Nestin, which are considered to be markers of cancer stem cells. Monolayer cells were similarly assayed for comparison. As shown in Figure 1B and C, 83.2% of cells in tumor spheres were CD133+, and 92.6% were Nestin+. We then tested whether the spheres were capable of multilineage differentiation. After culturing in DMEM/F12 containing 10% fetal bovine serum, the tumor spheres began to adhere to the bottom of culture plates, eventually spreading into monolayers and differentiating into cells expressing GFAP (Figure 1D).

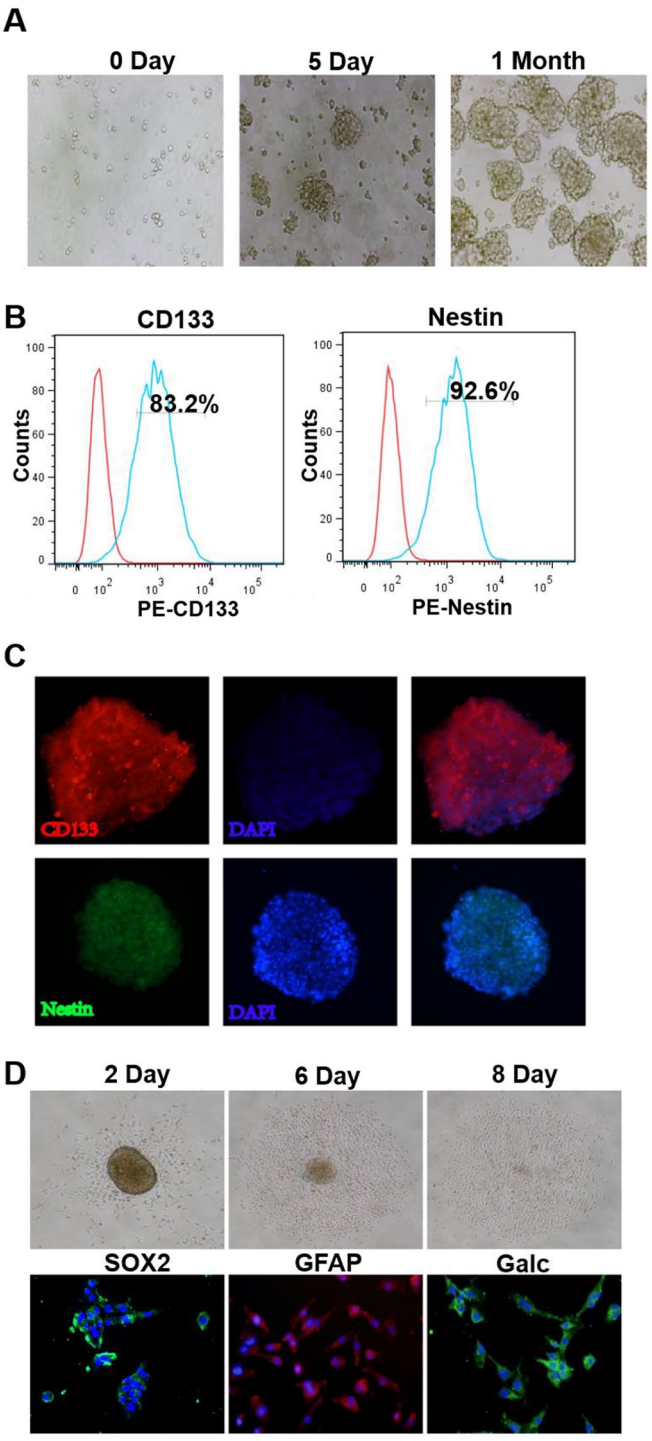

Figure 1. Isolation and differentiation of GSCs. (A) GL261 cells were cultured for 1 month and tumor spheres were observed by bright-field microscopy (10×). (B) CD133+ and Nestin+ cells were quantified by flow cytometry. (C) CD133+ (red) and Nestin+ (green) cells were observed by dark-field microscopy (20×). DAPI was used to stain nuclei (blue). (D) Spheres began to spread into monolayers after 2 days of culture, as observed under a bright field (10×). GFAP+, SOX2+, Galc+ cells were observed under a dark field (10×). Nuclei were stained with DAPI (blue).

Heat shock induces ICD in GL261 and GSCs

GL261 and GSCs were exposed to mild (42°C) or severe heat shock (47°C) for 1 h, and subsequently incubated at 37°C for

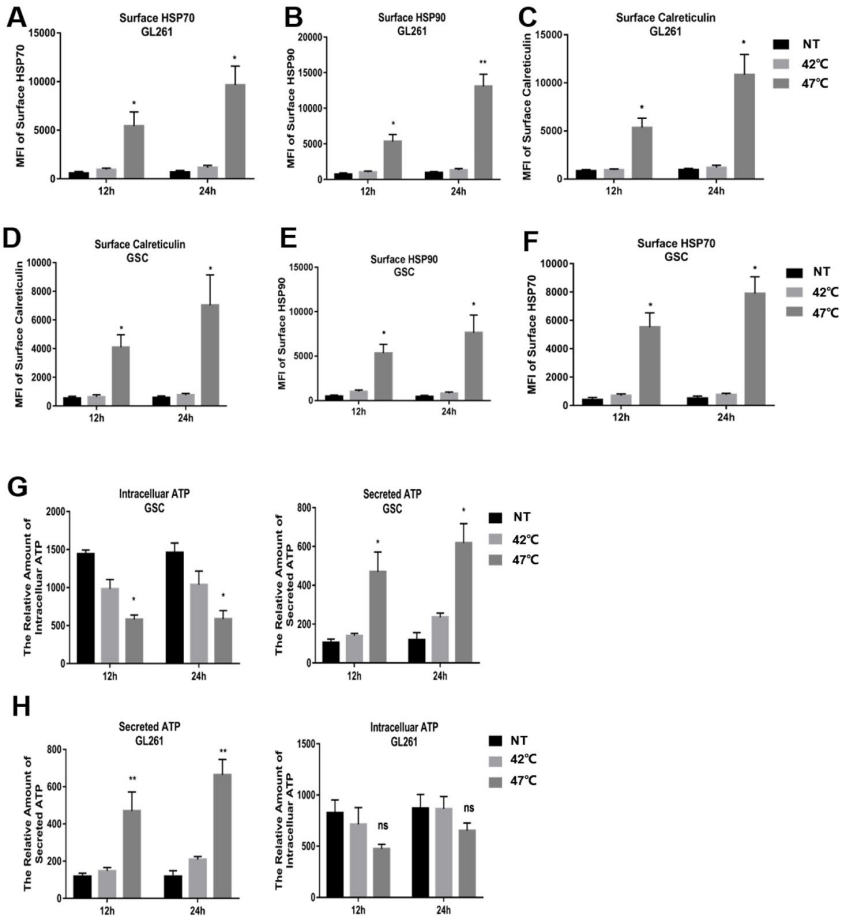

Figure 2. Heat shock induces major ICD associated DAMPs in murine GC (GL261) and GSC. GC and GSC were exposed to severe (47°C) or mild (42°C) heat shock to induce ICD. No treatment (NT) cells were used as control. Median fluorescent intensity from surface calreticulin, HSP70, and HSP90 were measured by flow cytometry in GC (A to C) and GSCs (D to F). The intracellular and secreted ATP were also detected in GC and GSCs (G, H). *$P < .05$; **$P < .01$ versus unheated cells.

12 h and 24 h. We found that heating at 47°C, but not at 42°C, induces cell surface exposure of calreticulin, HSP90, and HSP70 in both cells (Figure 2A to F). The intracellular and secreted ATP was also detected in GC and GSCs (Figure 2G and H). Collectively, the data suggest that in GL261 and GSCs, only severe heat shock induces molecular changes typical of ICD.

Heat-inactivated GL261 and GSCs activate DCs

Immature DCs were isolated from the bone marrow of C57BL/6 mice. These cells formed colonies after 3 days of culture and mature after stimulation with lipopolysaccharide. Thus, immature DCs were cultured for 6 days in the presence of GM-CSF and IL-4 and matured by addition of heat-inactivated GL261 or GSCs. We found that surface expression of CD40, CD80, CD86, and MHC-II significantly increased as result (Figure 3A). Furthermore, we found that DCs loaded with GL261 and GSC antigens abundantly release IL-6, IL-10, and TNF-α (Figure 3B to D) in comparison to naïve cells. Interestingly, DCs exposed to heat-inactivated GSCs secreted higher levels of IL-6 (Figure 3B) and TNF-α (Figure 3D) than cells loaded with apoptotic GL261 antigens. There was no significant difference of IL-10 (Figure 3C) and IFN-γ (Figure 3E) in expression between cells exposed to GL261 or GSCs.

Antigen-loaded DCs induce strong T-cell immunity and block stem cell-like behavior in live GSCs *in vitro*

T-cells were isolated from splenocytes of C57BL/6 mice by magnetic-activated cell sorting. These cells were then stimulated with DCs exposed to heat-inactivated GL261 and GSCs and assayed for cytotoxicity against live GL261 and GSCs. Results indicate that T-cells stimulated with antigen-loaded DCs were both superior in cytotoxicity to T-cells stimulated with immature DCs. In addition, cytotoxic activity was stronger in T-cells challenged with DCs loaded with GSC antigens, in comparison to T-cells challenged with DCs matured with GL261 antigens (Figure 4A).

The results showed that T-cells stimulated with antigen-loaded DCs also significantly reduced sphere formation (Figure 4B) and colony (Figure 4C) in live GL261 and GSCs. Migration and invasion were significantly impeded, as assessed by transwell and Boyden assays (Figure 4E and F).

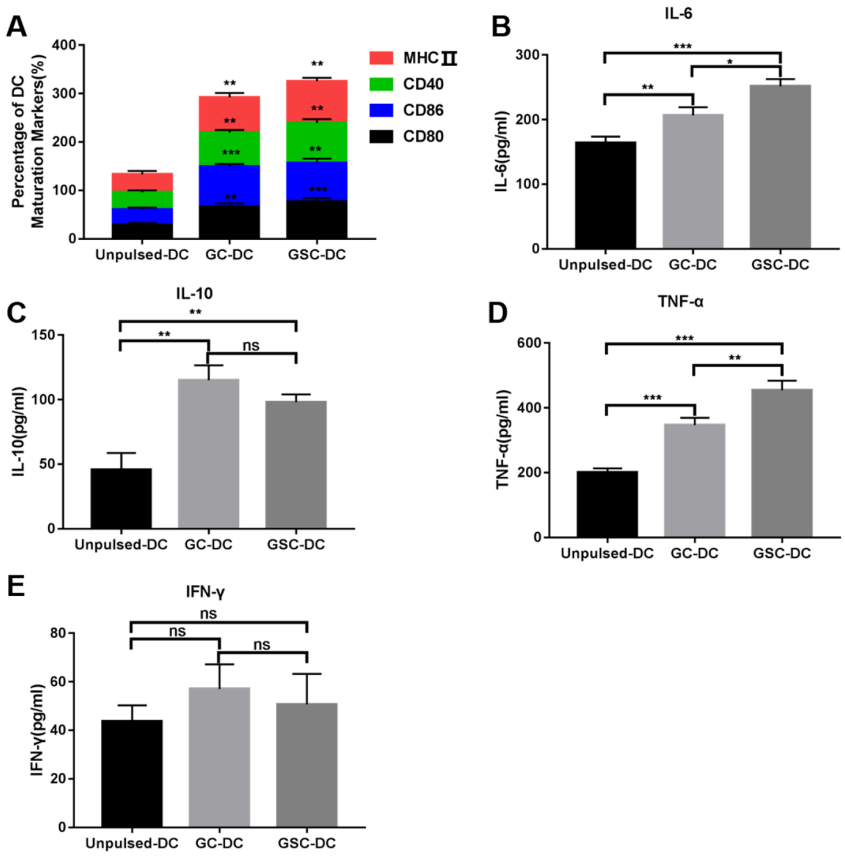

Figure 3. Heat shock-treated GC and GSC lysates activate DCs. (A) Immature DCs derived from the bone marrow progenitor cells of C57BL/6 mice were loaded with heat shock-inactivated GC and GSC lysates, and CD40+, CD80+, CD86+, and MHC II+ cells were quantified by flow cytometry. (B) Secretion of IL-6, (C) IL-10, (D) TNF-α, and (E) IFN-γ were analyzed by ELISA.
ns indicates no significant.
*$P < .05$; **$P < .01$; ***$P < .001$ versus DCs only.

Of note, T-cells stimulated with DCs exposed to GSC antigens have stronger inhibitory activities on all assays than T-cells stimulated with DCs exposed to heat-inactivated GL261 (Figure 4D). Collectively, the data provide in vitro evidence that DC vaccines prepared with GSCs elicit stronger antitumor activity than DC vaccines prepared with GL261.

Combination immunotherapy inhibited tumor growth and prolonged survival in an immunocompetent syngeneic glioma model

An overview of the treatment scheme for the glioma cell model is shown, respectively, in Figure 5A. Luciferase-expressing GL261 cells were transplanted into the brains of C57BL/6 mice. Tumor formation was verified using BLI. After a week, mice received an intraperitoneal injection of IgG, anti-CD47 antibody, DCs matured with GSC-DC vaccine, GSC-DC vaccine, and anti-CD47 antibody, respectively. Various symptoms of neurological deficits were also monitored in each group. The results showed that GSC-DC vaccine combined with anti-CD47 antibody group significantly inhibited tumor growth in vivo versus other groups (Figure 5B and C) and also prolonged survival time (Figure 5D).

Neurological deficit scores/grades during the experiment revealed a significant delay in the beginning of clinically relevant symptoms in GSC-DC vaccine combined with anti-CD47 antibody group (Figure 5G). Hematoxylin and eosin (H&E) staining of spleen tissue showed a marked expansion in the germinal centers of the combination treatment group (Figure 5F). Finally, an ELISA also indicated that combination treratment promoted the production of IFN-γ (Figure 5E).

Discussion

Because of their constitutive qualities and stemness activity, cancer stem cells are shielded from immunologic pressure. In addition, when cancer stem cells and other immune cell types work together, the immunological milieu becomes more suppressive, which encourages the progression of cancer.[24]

It would be beneficial to reduce tumor cell immune evasion and offer novel strategies to extend median life if combined with DC vaccinations.[25,26] As of right now, there are no medicinal approaches that effectively eradicate GSCs. Glioma stem cells recover quickly from traditional treatment stress because of their increased capacity, which causes resistance and ultimately relapse

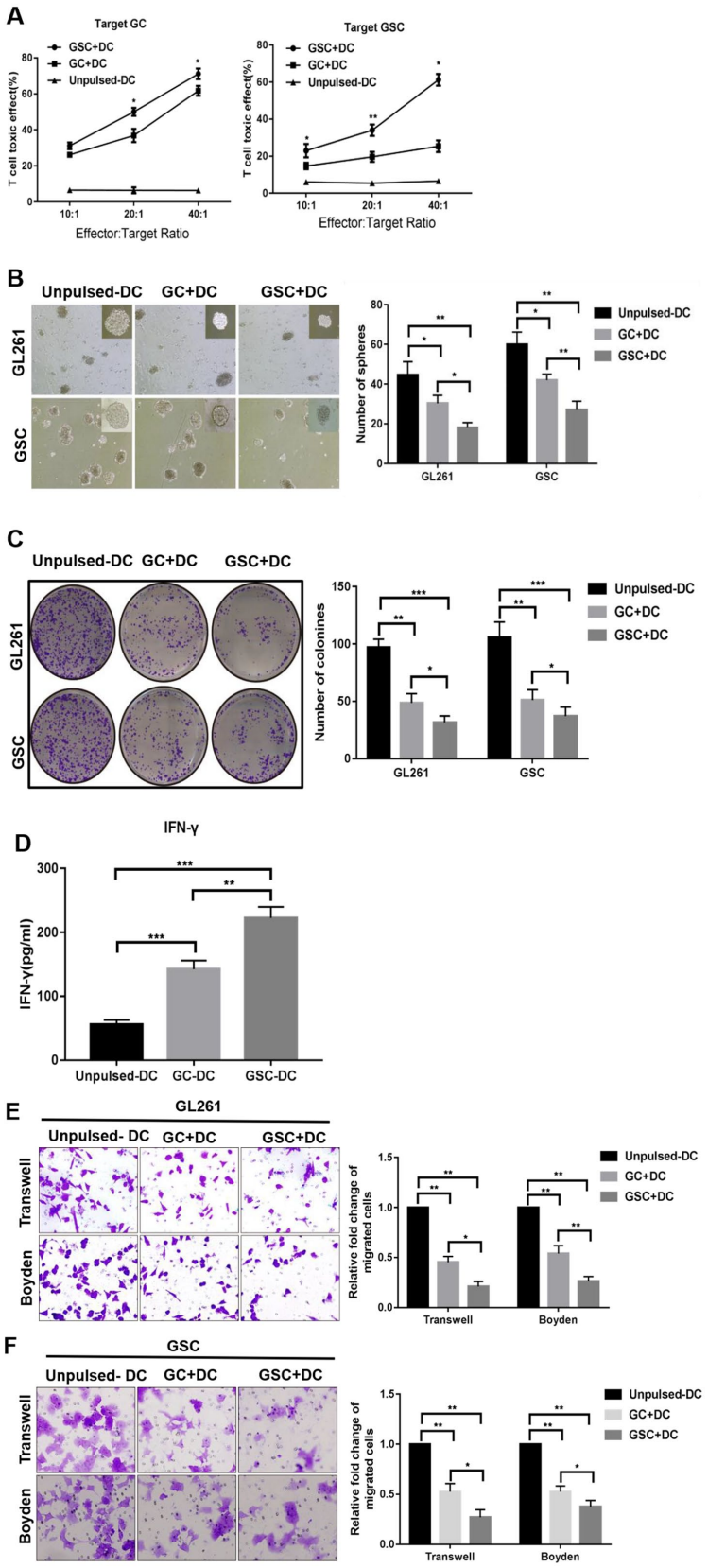

Figure 4. Dendritic cells loaded with heat shock-treated GSCs lysates significantly enhanced the GSC specific CTL activity diminish the characteristics of GSC in vitro. (A) Cytotoxicity of T-cells stimulated with dendritic cells that had been exposed to GC or GSCs. CTLs and target cells were incubated at ratios of 10:1, 20:1, and 40:1 as indicated. The cytotoxicity mediated by CTLs was measured by an LDH release assay (B) sphere formation and colony formation (C) of live GC and GSCs co-cultured with stimulated T-cells. (D) Secretion of IFN-γ was analyzed by ELISA. Migration and invasion were significantly impeded, as assessed by transwell (E) and Boyden assays (F).
*$P < .05$; **$P < .01$; ***$P < .001$.

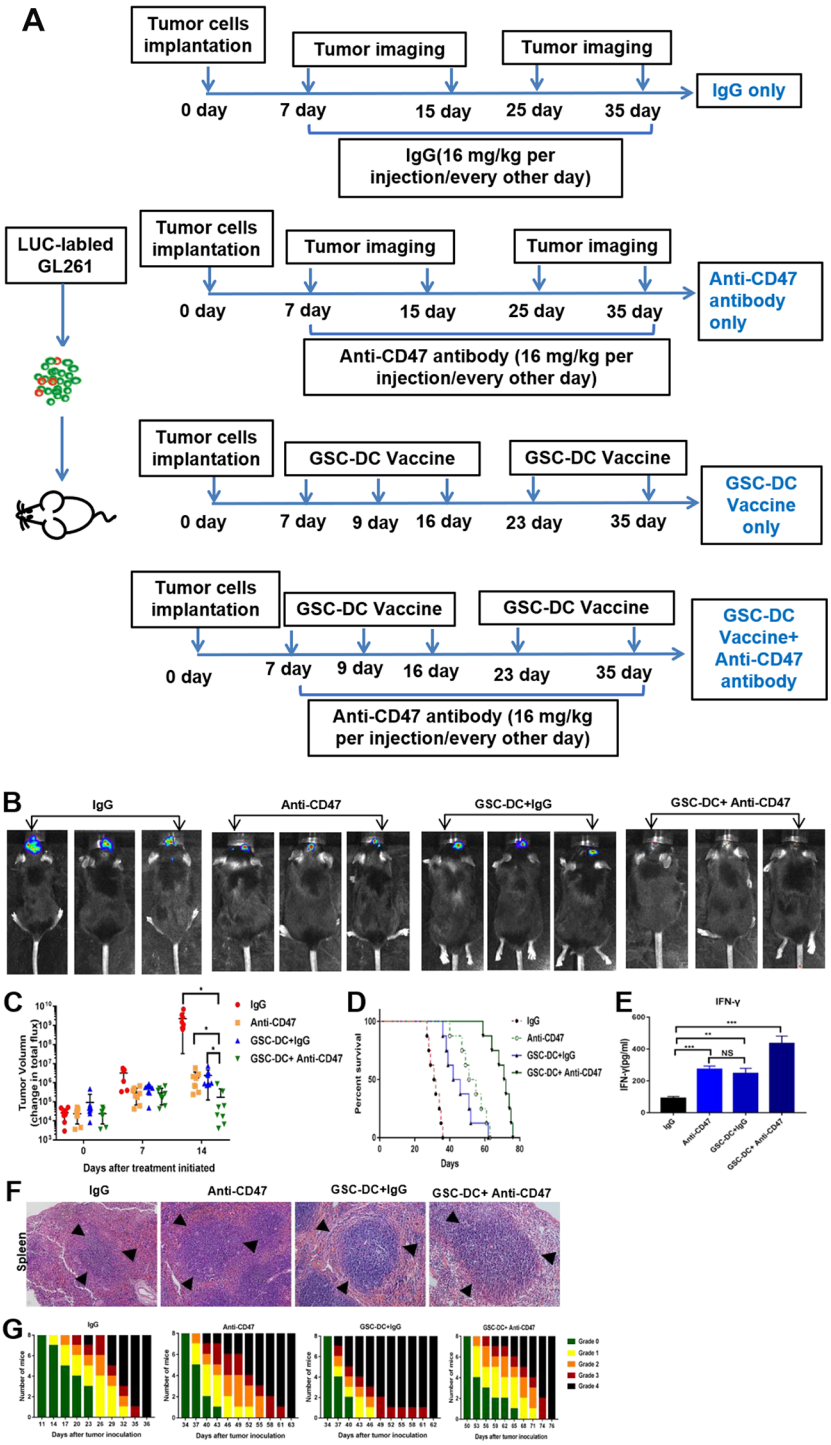

Figure 5. ICD-based DC vaccines, in combination with anti-CD47 mAb provide strong survival benefit in an immunocompetent syngeneic glioma model. (A) Treatment scheme of the GL261-implanted mice. (B) Representative BLI images and (C) measurements of tumor volume. (D) Combination therapy significantly prolonged survival time. (E) The production of IFN-γ was detected in peripheral blood by ELISA. (F) H&E staining of spleen tissue showed a marked expansion in the germinal centers of the GSC-DC vaccine in combination with anti-CD47 antibody treatment groups. (G) Tumor-induced neurological deficit symptoms over time. The grading scheme used was grade 0, healthy mice; grade 1, slight unilateral paralysis; grade 2, moderate unilateral paralysis and/or the beginning of a hunched back; grade 3, severe unilateral or bilateral paralysis and pronounced a hunched back; grade 4, moribund and/or dead mice.

in glioma patients. Immunogenic cell death has become a crucial element of therapy-induced antitumor immunity. Immunogenic cell death has been identified in recent years to be essential to many different new and established therapy approaches.[27] In this investigation, heat-inactivated GSCs increased DC maturation and cytokine production. The in vitro findings also showed that DC vaccines prepared with GSCs have a stronger anticancer effect than those made with glioma cell antigens.

Previous investigations demonstrated that, for solid tumors, a combination of immune intervention and local irradiation

was beneficial because the irradiated tumors enhanced the anticancer immune response.[28,29] To lower the tumor load and increase the patient's survival time, the first line of treatment for Glioblastoma (GBM) is currently surgical excision.[30] Then, to enhance the effects of antitumor DC vaccinations, combine DC vaccines with radiation therapy, chemotherapy, or both to induce DNA damage and endoplasmic reticulum stress, which will cause cell death and produce cytokines and hemokines that will augment DC stimulation signals.[31] According to our findings, severe heat shock caused apoptosis, ER stress, and the exposure of DAMP molecules on the cell surface and in the extracellular environment. Heat-inactivated GSCs enhanced the maturation of DCs and the proliferation of T lymphocytes. We previously reported that CD47+ glioma cells were highly enriched for GSCs, anti-CD47 mAb-inhibited tumor growth in an immunocompetent syngeneic GSC model.[23] Thus, ICD-based DC vaccines, in combination with anti-CD47 antibody were used in an immunocompetent syngeneic glioma model. However, despite the fact that the combination therapy has shown some promising therapeutic effects in human xenograft models, further research is necessary to determine the precise mechanism of action. A potential limitation of our study is that the combination treatment does not currently completely eradicate the glioma despite inhibiting tumor growth and prolonging survival.

In conclusion, these results provided credence to the idea that GSC-DC vaccines based on ICD could facilitate T lymphocytes recognize one other and overcome resistance to treatment. It is a potential approach to inhibit tumor formation and prevent tumor recurrence because adjuvant therapy enhances immune response in the GBM microenvironment by enhancing DAMPs exposure and release. Our study offered an effective immunotherapy strategy to demonstrate that a combination of the ICD-based DC vaccines, and anti-CD47 antibody therapy has the potential for tumor killing in vivo.

Conclusions

Long-term benefits of DC vaccination are uncertain of glioma patients. It is extremely important to develop new treatment strategies that enhance the immunotherapy effect of DC-based vaccines. Dendritic cells exposed to GSCs are considered promising vaccines against glioma. Heat shock induced ICD in GL261 and GSCs, as indicated by significant release of calreticulin, HSP70, HSP90. Heat-shock-conditioned ICD lysates induce maturation and activation-associated marker expression on monocyte-derived DCs. These results provided credence to the idea that ICD-based GSCDC vaccines provided considerable survival benefit against glioma. It is a potential approach to inhibit tumor formation and prevent tumor recurrence because adjuvant therapy enhances immune response in the GBM microenvironment by enhancing DAMPs exposure and release. The study offered an effective immunotherapy strategy to demonstrate that a combination of the ICD-based DC vaccines and anti-CD47 antibody therapy has the potential for tumor killing in vivo.

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Author Contributions

Feng Li and Xiaodan Jiang designed the study and assisted in the preparation of the manuscript. Qijia Tan drafted the manuscript. Jun Wang, Yi Liu, Yingqian Cai, Yuxi Zou conducted the experiments. All authors approved the version of the manuscript finally and agree to be accountable for the work.

Ethics Approval

This study complied with the Declaration of Helsinki and was approved by Ethical Committee of of Zhujiang hospital Southern Medical University (grant no. LAEC-2020-101) and conducted in accordance with the ethical standards.

Consent to Participate

Not applicable.

Data Availability

The data generated or analyzed during this study are included in this article.

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