Oncology and Translational Medicine
DOI 10.1007/s10330-022-0582-2

October 2022, Vol. 8, No. 5, P209–P216

ORIGINAL ARTICLE

Differential centrifugation enhances the anti-tumor immune effect of tumor lysate-pulsed dendritic cell vaccine against glioblastoma*

Xiaojin Liu, Yiwei Qi (Co-first author), Feng Hu, Kai Shu, Ting Lei (✉)

Sino-German Neuro-Oncology Molecular Laboratory, Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Abstract

Objective This study aimed to improve the antitumor immunocompetence of a tumor lysate-pulsed dendritic cell (DC) vaccine through differential centrifugation and provide a theoretical basis for its clinical application in glioblastoma.

Methods Peripheral blood mononuclear cells were extracted using Ficoll-Paque PLUS and induced into mature DCs *in vitro* with a cytokine cocktail. The modified tumor lysate was generated by differential centrifugation. The maturity markers of DCs in each group, namely the modified tumor lysate, tumor lysate, and negative and positive control groups, were assessed using flow cytometry. Furthermore, their ability to stimulate lymphocyte proliferation and *in vitro* antitumor effects were assessed using Cell Trace TM CFSE. IFN-γ secretion levels were measured with ELISA. Intracellular reactive oxygen species were measured using 2',7'-dichlorofluorescein diacetate (DCFDA) staining. The results were statistically analyzed using an unpaired Student's *t*-test and were considered significant at $P < 0.05$.

Results Compared with tumor lysate-pulsed DCs, modified tumor lysate-pulsed DCs had a higher expression of maturity markers: CD1a (7.38 ± 0.53% *vs*. 4.47 ± 0.75%) and CD83 (19.81 ± 4.09% *vs*. 9.64 ± 1.50%), were better capable of stimulating lymphocyte proliferation [proliferation index (PI): 8.54 ± 0.16 *vs*. 7.35 ± 0.05], secreting IFN-γ, and inducing stronger *in-vitro* cytotoxic T lymphocyte (CTL) cytotoxicity against glioblastoma cells. In addition, we found that the level of ROS in modified tumor lysate-pulsed DCs was lower than that in tumor lysate-pulsed DCs.

Conclusion Differential centrifugation of tumor lysates can improve the antitumor immunocompetence of DC vaccines, and reactive oxygen species may be the key to affecting DC function in the whole tumor lysate.

Received: 15 June 2022
Revised: 17 August 2022
Accepted: 22 September 2022

Key words: glioblastoma; immunotherapy; dendritic cell (DC) vaccine; reactive oxygen species

Glioblastoma is the most common primary malignant tumor of the central nervous system with an annual incidence rate of 3–5/100 000 and a poor prognosis of 14.6 months, accounting for about 50% of all gliomas [1–2]. Although there are numerous studies on glioblastoma, the anatomical location of the central nervous system limits the application of locoregional treatment. In addition, the tumor is characterized by endogenous radio- and chemoresistance, heterogeneity, and an immunosuppressive microenvironment; these factors limit the progress of glioblastoma treatment. The standard treatment is to safely maximize surgical resection with adjuvant radiotherapy and chemotherapy [3–4]. Other adjuvant treatments include tumor treating fields, immunotherapy, gene therapy, and molecular targeted therapy, among which immunotherapy has great potential in the treatment of glioblastoma.

As the most effective antigen-presenting cells, dendritic cells (DCs), bridge the innate and adaptive immune system [5–7]. DCs are cultured *in vitro* for maturation, sensitized with tumor-associated antigens or tumor-specific antigens, and then injected back into

✉ Correspondence to: Ting Lei. Email: tlei@tjh.tjmu.edu.cn
* Supported by the National Natural Science Foundation of China (No. 81602202 and 82173136).

the patient to elicit an antitumor immune response [8–11]. Many clinical studies have shown that DC vaccines are safe and rarely have autoimmune-related side effects, but their clinical efficacy remains limited [12–15]. Therefore, maximizing the effectiveness of DC vaccines is a hot topic in the field of immunotherapy. Considering the tumor heterogeneity of glioblastoma, it is difficult to induce an effective antitumor immune response by sensitizing DCs with a single antigen. Although tumor lysates contain multiple epitopes of tumor antigens, many components released from tumor cell lysis may inhibit DC maturation, thereby affecting their ability to effectively present antigens. Most studies have improved the anti-tumor immune effect of the DC vaccine through combination with immune adjuvants [16–18] but few have attempted to eliminate the possible components of whole tumor lysate that may inhibit the maturation of DCs to improve the clinical efficacy of DC vaccines.

Reactive oxygen species is a collective term for the superoxide anion (O_2^-), hydroxyl radical (HO•), and hydrogen peroxide (H_2O_2), which are mainly released from the electron transport chain in the mitochondria and membrane-bound NADPH oxidase complexes (NOXs). They are involved in the pathogenesis of many diseases such as tumors, aging, diabetes, neurodegenerative diseases, and atherosclerosis [19]. Many studies have shown that reactive oxygen species have a sophisticated effect on the function of DCs and can elicit bidirectional regulation of their maturation. Studies have shown that the reactive oxygen species in the tumor microenvironment can induce endoplasmic reticulum stress in ovarian tumor-associated DCs, causing abnormal deposition of lipid peroxides in DCs, resulting in a decrease in their ability to activate T cells, leading to immune tolerance [20]. Oxidized lipid products can affect antigen presentation by DCs, subsequently compromising their ability to stimulate adaptive immunity [21–22].

Differential centrifugation is a well-developed biological method for separating organelles, through which organelles with differences in sedimentation coefficients are separated from each other. We attempted to sequentially remove the main sources of reactive oxygen, including mitochondria, lysosomes, and peroxisomes, from the whole tumor lysate through differential centrifugation, reducing the level of reactive oxygen species in DCs sensitized by tumor lysate to boost their maturity. We found that modified tumor lysate-pulsed DCs have a higher expression of surface markers, such as CD1a and CD83, which are more capable of stimulating lymphocytes and inducing stronger *in vitro* cytotoxic T lymphocyte (CTL) cytotoxicity against glioblastoma cells.

Materials and methods

Cell culture

The glioma cell lines U87 and U251 were cultured in DMEM supplemented with 10% FBS, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/mL streptomycin.

In vitro generation of human monocyte-derived DCs

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donor blood using Ficoll-Paque PLUS according to the manufacturer's instructions. The cells were incubated for 2 h at 37 °C at a concentration of 5×10^6 cells/mL in RPMI 1640 (Gibco, NY, USA). Non-adherent cells were removed, and adherent PBMCs were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, recombinant human granulocyte-macrophage colony-stimulating factor /rhGM-CSF (800 units/mL; R&D system, MN, USA), and recombinant human interleukin (IL)-4 /rhIL-4 (400 units/mL; R&D). DCs were activated by lipopolysaccharide (LPS; 0.5 μg/mL) and TNF-α (200 units/mL).

Tumor lysate preparation and DC loading

Glioma cell lines were harvested, washed twice in PBS, and resuspended at a density of 2×10^6 cells/mL in serum-free medium. The cell suspensions were frozen at 80 °C and thawed at 37 °C for four freeze-thaw cycles. To remove cell debris, the lysate was centrifuged at 1000 × *g* for 10 min. The supernatant was collected as a classic tumor lysate and passed through a 0.2 μm filter. The modified tumor lysate was prepared by differential centrifugation. Briefly, the lysate was centrifuged at 1000 × *g* for 10 min to remove debris and cell nuclei, the supernatant centrifuged at 10000 × *g* for 10 min to remove mitochondria, then at 15300 × *g* for 20 min to remove lysosomes and peroxisomes. Finally, the supernatant was collected as a modified tumor lysate containing microvesicles and soluble proteins. The protein concentration of the lysate was determined using the BCA kit (NCM Biotech, Suzhou, China). DCs were pulsed with the tumor lysate and modified tumor lysate at a concentration of 100 μg/mL.

Flow cytometric analysis

DCs and lymphocytes were removed from the plate and centrifuged at 1500 × *g* for 5 min. The pelleted cells were washed with RPMI-1640 and incubated at 4 °C for 30 min with FITC-anti-CD1a (catalog No. 300103), FITC-anti-CD83 (catalog No. 305305), PE/CY5-anti-CD86 (catalog No. 305407), PE-anti-HLA-DR (catalog No. 307605), and PE-anti-CD3 (catalog No. 300309), PE-anti-CD4 (catalog No. 317409), FITC-CD8 (catalog No. 344703). Species-

and isotype-matched monoclonal antibodies were used as controls. All antibodies were purchased from BioLegend, San Diego, CA, USA. The cells were washed twice with PBS and analyzed using a FACSort flow cytometer (BD Bioscience, San Jose, CA, USA). Flow cytometry data were analyzed using FlowJo v.9 (TreeStar, Ashland, OR).

Proliferative T cell response and cytokine measurement

Human blood samples were collected from healthy donors under the principles of the ethical committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China. PBMCs were isolated by Ficoll-Paque PLUS density gradient centrifugation and depleted of red blood cells using ammonium chloride. The cells were incubated for 2 h at 37 °C at a concentration of 5×10^6 cells/mL in RPMI 1640 (Gibco, NY, USA). Non-adherent cells were collected and cultured in RPMI 1640 medium supplemented with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 100 U/mL IL-2 (R&D system, MN, USA). These cells were co-cultured with autologous DCs in four parallel groups consisting of PBS-pulsed, LPS-pulsed, tumor lysate-pulsed, or modified tumor lysate-pulsed DCs at a ratio of 10:1 in RPMI 1640 culture medium containing IL-2 (50 U/mL). On day 5, the culture media were collected for the measurement of IFN-γ secretion using an ELISA kit (Proteintech, Rosemont, IL, USA). The capacity of the DC-induced proliferative T cell response was determined using Cell Trace™ CFSE (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions.

Determination of CTL cytotoxicity

After DC-T cell co-culture, the cells were collected as effector cells on day 5. The glioma cell lines U87 and U251 were labeled with CFSE (Invitrogen, Waltham, MA, USA), cultured in 6-well plates, and used as target cells for cytotoxicity assays. After 24 h, the medium was removed, and effector cells were added to CFSE-labeled target cells at an E:T ratio ["E" means effector cells (CTLs), "T" means target cells (U87 and U251)] of 50:1 in RPMI 1640 supplemented with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 100 U/ml IL-2 (R&D system, MN, USA).

Measurement of ROS

Intracellular ROS was measured using 2',7'-dichlorofluorescein diacetate (DCFDA) staining (Abcam, Cambridge, UK) according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software Inc., CA, USA). Differences between the means of experimental groups were statistically compared using unpaired Student's *t*-test, and significance was set at $P < 0.05$.

Results

DC morphology and expression of surface markers

Human blood mononuclear cells were induced and cultured *in vitro*. Small adherent round mononuclear cells and precursor cells were visible on day 2 under an inverted phase contrast microscope (Fig. 1a) and had a low expression of surface molecules such as CD1a (2.78 ± 0.88%), CD83 (3.74 ± 0.93%), and CD86 (50.70 ± 0.98%) (Fig. 1c and 1e). As the stimulation time increased, the cells became irregular, bulged, and larger. Around the 5th day, DC colonies began to form. The cell volume continued to grow and the bulges became more prominent. At this time, the cells remained immature. After stimulation with LPS and TNF-α for 24–48 h, the DCs were gradually separated from the colonies, presenting an irregular morphology of increased volume and elongated bulge. The PBMCs were finally induced into mature irregular DCs with clear protrusions *in vitro* on day 7 (Fig. 1b), which had a higher expression of CD1a (7.43 ± 0.44%), CD83 (18.16 ± 5.91%), and CD86 (92.35 ± 2.56%) (Fig. 1d and 1e).

Upregulation of maturation markers on modified tumor lysate-primed DCs

Through differential centrifugation, the tumor lysate was modified to contain microvesicles and soluble proteins (Fig. 2a). DCs were loaded with tumor lysate, modified tumor lysate, PBS (negative control group), and LPS (positive control group) on day 5. The expression of surface markers on DCs stimulated by different antigens was analyzed by flow cytometry on day 7. The result showed that the modified tumor lysate-pulsed DCs had a significantly higher expression of CD1a (7.38 ± 0.53% *vs.* 4.47 ± 0.75%) and CD83 (19.81 ± 4.09% *vs.* 9.64 ± 1.50%), compared with that of the tumor lysate group. CD86 (92.07 ± 0.355% *vs.* 91.72 ± 1.748%) and HLA-DR (98.04 ± 2.02% *vs.* 95.19 ± 2.06%) remained at a high level, and there was no statistical significance between the two groups (Fig. 2b).

Modified tumor lysate-pulsed DCs stimulated lymphocyte proliferation and secretion of IFN-γ

Lymphocytes were successfully isolated from PBMCs and identified using flow cytometry (Fig. 3a–3c). To measure proliferative T cell responses, we co-cultured T cells with DCs for 1, 3, and 5 d. We found that T cells

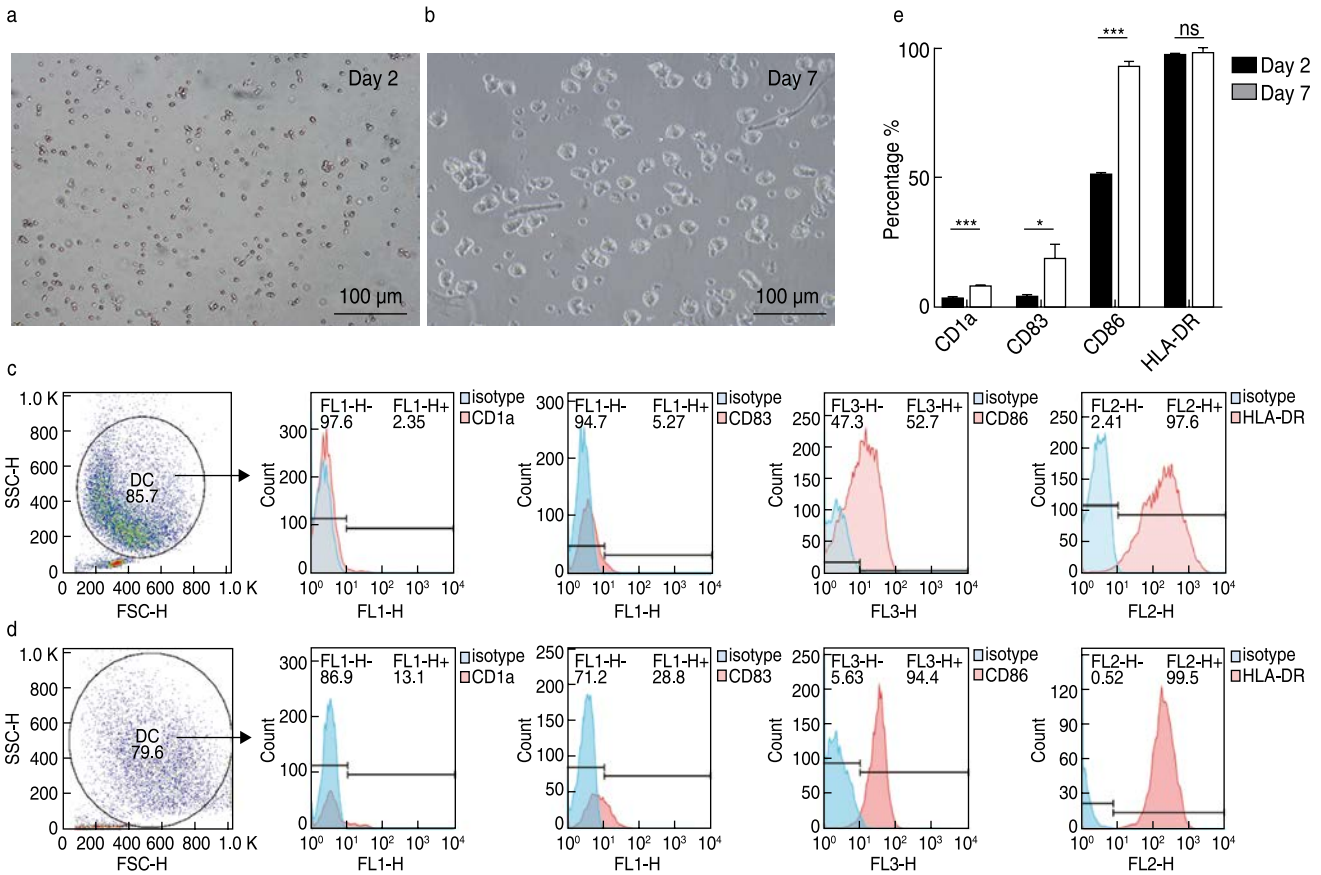

Fig. 1 Dendritic cell (DC) morphology and surface markers. (a) and (b) show DC morphology on Day 2 and Day 7, respectively. (c) and (d) represent the expression of DC surface markers (CD1a, CD83, CD86, and HLA-DR) on Day 2 and Day 7, respectively. (e) represents the statistic histogram of the expression of DC surface markers. Scale bar = 100 μm. * $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$

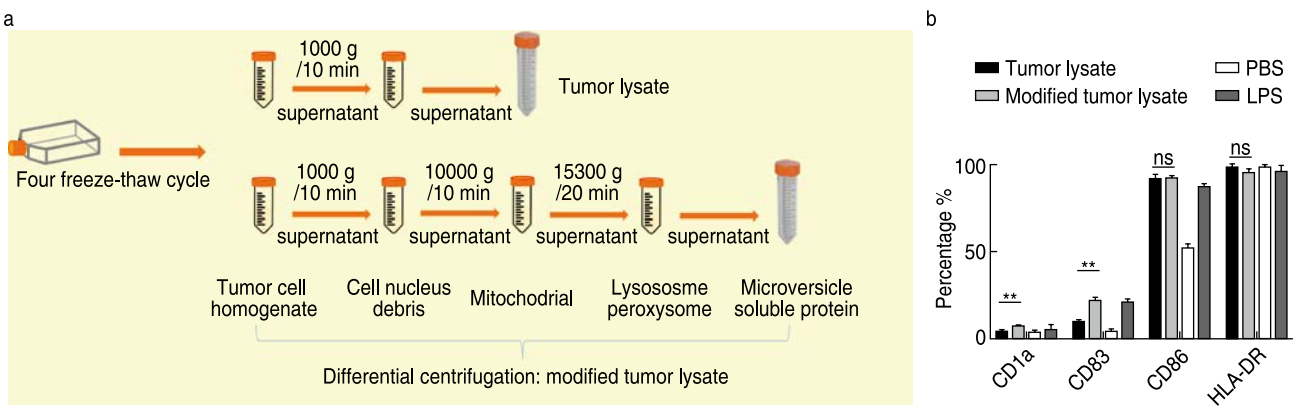

Fig. 2 Upregulation of maturation markers in modified tumor lysate-primed DCs. (a) illustrates the preparation of the modified tumor lysate through differential centrifugation. (b) shows that modified tumor-lysate-primed DCs have a higher expression of maturation markers. ** $P < 0.01$

were not activated and began to proliferate after 1 and 3 d of co-culture, and no differences between these four co-culture groups in terms of proliferation index, compared to T cells alone were found (Fig. 3d and 3e). After 5 d of co-culture, we showed that modified tumor lysate-pulsed DCs were better capable of stimulating T cells and triggered stronger proliferative responses than that of the other three groups (Fig. 3f and 3g). Furthermore, we confirmed that T cells had a higher ratio of $CD8^+$ / $CD4^+$ cells after co-culture with DCs; however, there

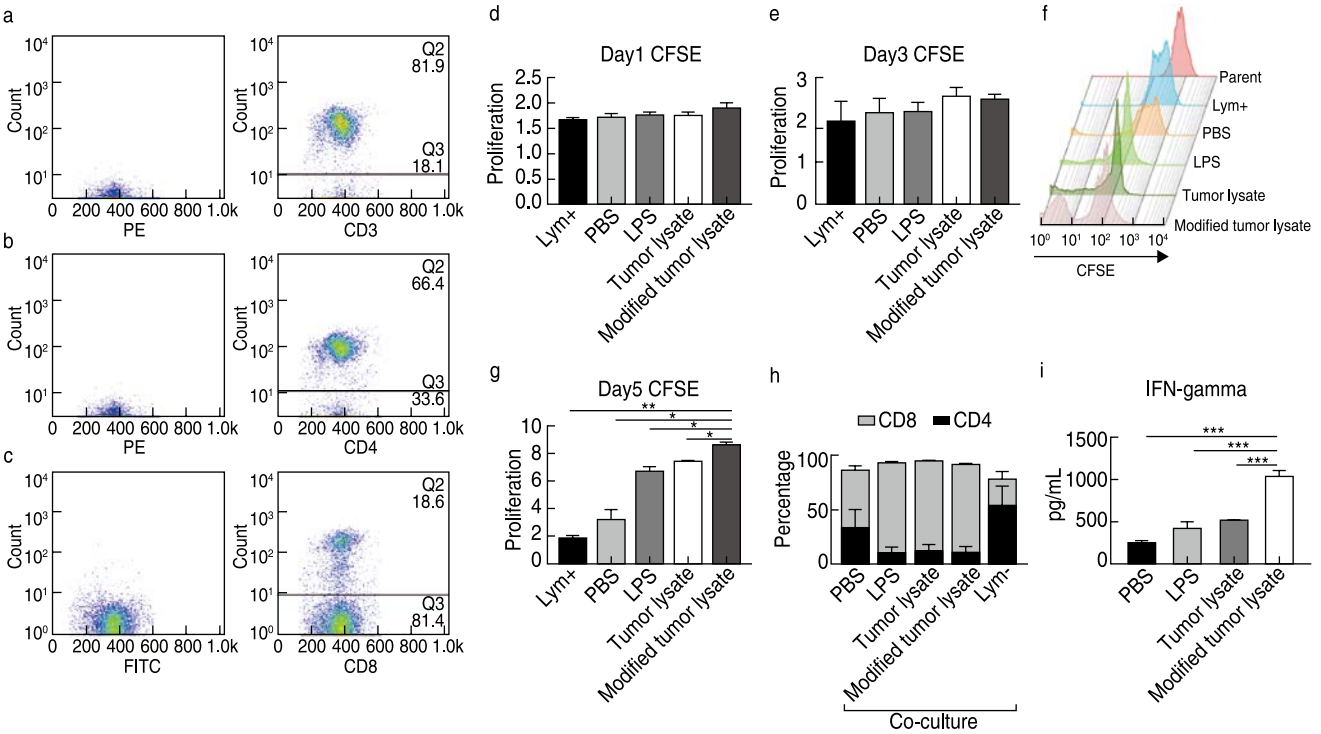

Fig. 3 T cell proliferation and IFN-γ secretion. (a), (b), and (c) show the flow cytometry scatter plot of CD3, CD4, and CD8, respectively on lymphocytes isolated from PBMCs. (d) and (e) represent the statistical histogram of the proliferative T cell responses on Day 1 and Day 3, respectively, after co-culture with tumor lysate, modified tumor lysate, PBS, and LPS pulsed DCs. (f) and (g) exhibit the flow cytometry overlay histogram plot and statistical histogram, respectively, which represents the proliferative T cell response on Day 5 after co-culture with the four groups of DCs. Lym+ stands for the group of T cells without co-culture. (h) indicates the ratio of $CD8^+/CD4^+$ T cells after co-culture with the four groups of DCs. Lym– stands for the group of T cells before co-culture. (i) represent the statistical histogram of the IFN-γ secretion level in the four groups. * $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$

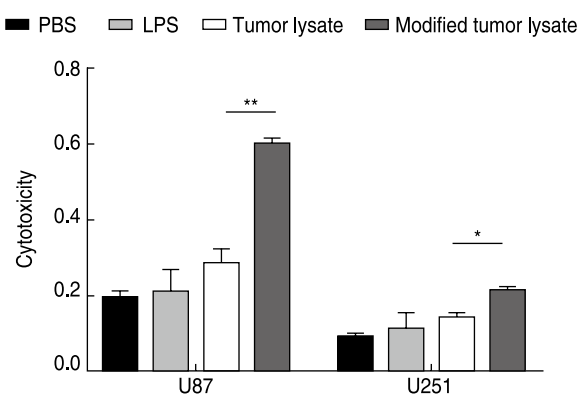

Fig. 4 Cytotoxicity of CTLs pulsed with DC vaccines. CTLs pulsed with modified tumor lysate-primed DCs showed the strongest cytotoxicity against tumor cells (U87 and U251). * $P < 0.1$; ** $P < 0.01$

were no differences between tumor lysate-pulsed DCs and modified tumor lysate-pulsed DCs in terms of the ratio of $CD8^+/CD4^+$ T cells (Fig. 3h). After co-culture of T cells with DCs for 5 d, we collected the culture media and measured the secretion level of IFN-γ using an ELISA kit. We demonstrated that the level of IFN-γ was significantly higher in the modified tumor lysate-pulsed DCs than that in the tumor lysate, PBS, or LPS-pulsed DCs (Fig. 3i).

Modified tumor lysate-pulsed DCs induced CTL cytotoxicity against glioblastoma cells

To determine the cytotoxicity of T cells stimulated by modified tumor lysate-pulsed DCs, we used the CFSE-labeled glioma cell lines U87 and U251 as target cells. The cytotoxicity of T cells activated by modified tumor lysate-pulsed DCs was significantly greater than that of PBS, LPS, and tumor lysate-pulsed DCs (Fig. 4).

ROS in modified tumor lysate-pulsed DCs

To explore the mechanism involved in the improvement of the *in vitro* antitumor immunocompetence of the modified DC vaccine, we measured ROS in the tumor lysate and modified tumor lysate-pulsed DCs. We found that the level of ROS in modified tumor lysate-pulsed DCs was lower than that in tumor lysate-pulsed DCs (Fig. 5).

Discussion

Immunotherapy is one of the most promising adjuvant treatments for glioblastoma following surgery, radiotherapy, and chemotherapy. DC vaccines play vital

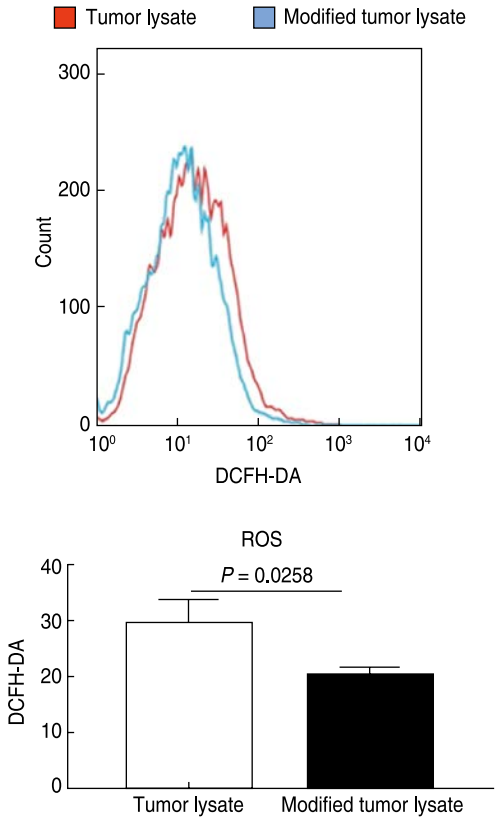

Fig. 5 ROS in different lysate-primed DCs

roles in immunotherapy [23–24]. The high heterogeneity of glioblastomas limits the antitumor effect of a single antigen-sensitized DC vaccine. Although the whole tumor lysate contains multiple epitopes of tumor antigens, many components released during cell lysis may affect the normal function of DCs. This study highlights that by using differential centrifugation to sequentially remove the sources of ROS, including mitochondria, lysosomes, and peroxisomes, from the whole tumor lysate, we increased the maturity of DCs and their ability to stimulate lymphocyte proliferation and secrete IFN-γ. Meanwhile, the number of $CD8^+$ lymphocytes increased, contributing to a more effective antitumor immune response.

The maturity of DCs determines their ability to stimulate lymphocytes and their antitumor effects. Mature DCs can elicit an effective adaptive immune response, whereas immature DCs induce immune tolerance [25–26]. In this study, differential centrifugation was used to remove the source of ROS, which may affect the maturation of DCs from the whole tumor lysate. We showed that the modified tumor lysate-pulsed DCs had a higher expression of surface markers such as CD83 and CD1a, and a better ability to stimulate lymphocyte proliferation and IFN-γ secretion, which indicates a more efficacious adaptive immune response. The cross-presentation of foreign antigens by DCs to $CD8^+$ T lymphocytes underpins anti-tumor immunity [23, 27]. Chen *et al* proposed that DCs present foreign antigens to naive $CD8^+$ T cells through cross-activation in the form of MHC-I-antigen complexes and stimulate them into CTLs, which exert a strong and specific anti-tumor immune response [28]. Studies have shown that infiltrating T lymphocytes in the tumor microenvironment, especially $CD8^+$ T cells, are closely related to the anti-tumor immune effect, and a higher infiltration of T cells suggests a relatively better prognosis for patients with malignant tumors [29–30]. The modified DC vaccine prepared in this study triggered an evident lymphocyte ratio conversion after co-culture with lymphocytes *in vitro*. There was no significant difference in the ratio of $CD8^+/CD4^+$ lymphocytes between the tumor lysate and modified tumor lysate-pulsed DCs; nonetheless, the latter was better capable of stimulating the proliferation of lymphocytes. Therefore, the number of $CD8^+$ T cells stimulated by the modified tumor lysate-pulsed DCs was considerably increased, indicating that the modified DC vaccine can trigger a more powerful anti-tumor immune response. We further confirmed that the modified DC vaccine was more effective in triggering CTL cytotoxicity against glioma cells.

Many studies have shown that the effect of ROS on DC function is complex. The maturation and antigen-presenting ability of DCs are bidirectionally regulated through different mechanisms [31–33]. Rutault *et al* found that the maturity of DCs increased after H_2O_2 intervention [34]. However, studies have shown that reactive oxygen species inhibit the maturation of DCs by inducing endoplasmic reticulum stress [35]. Herber *et al* found that the abnormal deposition of oxidized liposomes in DCs reduced their ability to process and present tumor-related antigens, compromising their ability to trigger anti-tumor immune responses, which was restored after the reduction of oxidized liposomes through drug intervention in lipid synthesis [36]. Moreover, Herrera *et al* showed that abnormal oxidative stress in breast cancer patients indicates an increased risk of tumor recurrence [37]. In 2015, Juan *et al* confirmed that ROS in the tumor microenvironment inhibits the ability of DCs to stimulate T cells by inducing abnormally activated endoplasmic reticulum stress in ovarian tumor-associated DCs. Intervention in the endoplasmic reticulum stress signaling pathway restores the ability of tumor-associated DCs to activate T cells and elicit efficacious anti-tumor immune responses [20]. As one of the main sources of ROS, mitochondria play a key role in maintaining cellular redox balance [38]. Studies have shown that mitochondria continue to release ROS for 48 h, even after dysfunction of the electron transport chain [39]. Chougnet *et al* confirmed that mitochondrial-derived ROS inhibit the ability of

bone marrow-derived DCs to cross-present antigens in mice [32]. In this study, the main sources of ROS, including mitochondria, lysosomes, and peroxisomes, from the whole tumor lysate without a drug intervention, eliminated possible components that may inhibit the function of DCs through differential centrifugation. We found that the modified tumor lysate-pulsed DCs had a significant decrease in the level of intracellular ROS, which may explain, to a certain extent, the mechanism by which the modified tumor lysate stimulates DCs to further mature and trigger a more effective anti-tumor immune response.

Nevertheless, this study has some limitations and provides direction for further exploration and improvement. First, all experiments were *in vitro* cell experiments, which cannot reflect the role of the immune system as a whole and the impact of the tumor microenvironment. Second, although this study detected a decrease in the level of ROS in DCs sensitized by modified tumor lysate, we could not clarify whether ROS are involved in the mechanism of the modified DC vaccine to elicit more effective antitumor immune responses. Therefore, further studies are required. Third, considering that the components of the whole tumor lysate are complex, it is still necessary to further clarify which specific components affect the anti-tumor immune activity of the whole tumor lysate-sensitized DC vaccine.

Conclusion

In summary, this study improved the *in vitro* antitumor immunocompetence of the whole tumor lysate-pulsed DC vaccine against glioblastoma through differential centrifugation without a drug intervention. Meanwhile, we proposed that reactive oxygen species may be the key to affecting DC function in whole tumor lysates, providing a theoretical basis for the clinical application of a modified tumor lysate-pulsed DC vaccine in glioblastoma.

Acknowledgments

Not applicable.

Funding

Supported by the National Natural Science Foundation of China (No. 81602202 and 82173136).

Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

All authors contributed to data acquisition and interpretation and reviewed and approved the final version of this manuscript.

Data availability statement

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Ethical approval

This study was conducted in accordance with the standards of the Human Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China.

References

1. Ostrom QT, Gittleman H, de Blank PM, et al. American Brain Tumor Association adolescent and young adult primary brain and central nervous system tumors diagnosed in the United States in 2008–2012. Neuro Oncol. 2016;18(Suppl 1):i1-i50.
2. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008–2012. Neuro Oncol. 2015;17(Suppl 4):iv1-iv62.
3. Batash R, Asna N, Schaffer P, et al. Glioblastoma multiforme, diagnosis and treatment; recent literature review. Curr Med Chem. 2017;24(27):3002-3009.
4. Bush NA, Chang SM, Berger MS. Current and future strategies for treatment of glioma. Neurosurg Rev. 2017;40(1):1-14.
5. Poltorak MP, Schraml BU. Fate mapping of dendritic cells. Front Immunol. 2015;6:199.
6. Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology. 2018;154(1):3-20.
7. Qian C, Cao X. Dendritic cells in the regulation of immunity and inflammation. Semin Immunol. 2018;35:3-11.
8. Filley AC, Dey M. Dendritic cell based vaccination strategy: an evolving paradigm. J Neurooncol. 2017;133(2):223-235.
9. Garg AD, Coulie PG, Van den Eynde BJ, et al. Integrating next-generation dendritic cell vaccines into the current cancer immunotherapy landscape. Trends Immunol. 2017;38(8):577-593.
10. Saxena M, Bhardwaj N. Re-emergence of dendritic cell vaccines for cancer treatment. Trends Cancer. 2018;4(2):119-137.
11. Wang Y, Xie RF, Niu HQ, et al. IL-13Ra2- and glioma stem cell-pulsed dendritic cells induce glioma cell death *in vitro*. Oncol Transl Med. 2016;2(5):210-215.
12. Phuphanich S, Wheeler CJ, Rudnick JD, et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer Immunol Immunother. 2013;62(1):125-135.
13. Vik-Mo EO, Nyakas M, Mikkelsen BV, et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunol Immunother. 2013;62(9):1499-1509.
14. Constantino J, Gomes C, Falcão A, et al. Antitumor dendritic cell-based vaccines: lessons from 20 years of clinical trials and future perspectives. Transl Res. 2016;168:74-95.
15. Liau LM, Ashkan K, Tran DD, et al. First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med. 2018;16(1):142.
16. Lee AW, Truong T, Bickham K, et al. A clinical grade cocktail of cytokines and PGE2 results in uniform maturation of human monocyte-derived dendritic cells: implications for immunotherapy. Vaccine. 2002;20 Suppl 4:A8-A22.

17. Boullart AC, Aarntzen EH, Verdijk P, et al. Maturation of monocyte-derived dendritic cells with Toll-like receptor 3 and 7/8 ligands combined with prostaglandin E2 results in high interleukin-12 production and cell migration. Cancer Immunol Immunother. 2008;57(11):1589-1597.
18. Van Lint S, Wilgenhof S, Heirman C, et al. Optimized dendritic cell-based immunotherapy for melanoma: the TriMix-formula. Cancer Immunol Immunother. 2014;63(9):959-967.
19. Chio IIC, Tuveson DA. ROS in cancer: the burning question. Trends Mol Med. 2017;23(5):411-429.
20. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell. 2015;161(7):1527-1538.
21. Ramakrishnan R, Tyurin VA, Veglia F, et al. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. J Immunol. 2014;192(6):2920-2931.
22. Veglia F, Tyurin VA, Mohammadyani D, et al. Lipid bodies containing oxidatively truncated lipids block antigen cross-presentation by dendritic cells in cancer. Nat Commun. 2017;8(1):2122.
23. Le Gall CM, Weiden J, Eggermont LJ, et al. Dendritic cells in cancer immunotherapy. Nat Mater. 2018;17(6):474-475.
24. Lim M, Xia Y, Bettegowda C, et al. Current state of immunotherapy for glioblastoma. Nat Rev Clin Oncol. 2018;15(7):422-442.
25. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol. 1991;9:271-296.
26. Steinman RM, Nussenzweig MC. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. Proc Natl Acad Sci USA. 2002;99(1):351-358.
27. Kurts C, Robinson BW, Knolle PA. Cross-priming in health and disease. Nat Rev Immunol. 2010;10(6):403-414.
28. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1-10.
29. Fridman WH, Pagès F, Sautès-Fridman C, et al. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298-306.
30. Reiser J, Banerjee A. Effector, memory, and dysfunctional CD8(+) T cell fates in the antitumor immune response. J Immunol Res. 2016;2016:8941260.
31. Paardekooper LM, Vos W, van den Bogaart G. Oxygen in the tumor microenvironment: effects on dendritic cell function. Oncotarget. 2019;10(8):883-896.
32. Chougnet CA, Thacker RI, Shehata HM, et al. Loss of phagocytic and antigen cross-presenting capacity in aging dendritic cells is associated with mitochondrial dysfunction. J Immunol. 2015;195(6):2624-2632.
33. Dingjan I, Paardekooper LM, Verboogen DRJ, et al. VAMP8-mediated NOX2 recruitment to endosomes is necessary for antigen release. Eur J Cell Biol. 2017;96(7):705-714.
34. Rutault K, Alderman C, Chain BM, et al. Reactive oxygen species activate human peripheral blood dendritic cells. Free Radic Biol Med. 1999;26(1-2):232-238.
35. Luís A, Martins JD, Silva A, et al. Oxidative stress-dependent activation of the eIF2α–ATF4 unfolded protein response branch by skin sensitizer 1-fluoro-2,4-dinitrobenzene modulates dendritic-like cell maturation and inflammatory status in a biphasic manner [corrected]. Free Radic Biol Med. 2014;77:217-229.
36. Herber DL, Cao W, Nefedova Y, et al. Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 2010;16(8):880-886.
37. Herrera AC, Victorino VJ, Campos FC, et al. Impact of tumor removal on the systemic oxidative profile of patients with breast cancer discloses lipid peroxidation at diagnosis as a putative marker of disease recurrence. Clin Breast Cancer. 2014;14(6):451-459.
38. Angajala A, Lim S, Phillips JB, et al. Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. Front Immunol. 2018;9:1605.
39. Qian W, Kumar N, Roginskaya V, et al. Chemoptogenetic damage to mitochondria causes rapid telomere dysfunction. Proc Natl Acad Sci USA. 2019;116(37):18435-18444.

DOI 10.1007/s10330-022-0582-2
Cite this article as: Liu XJ, Qi YW, Hu F, et al. Differential centrifugation enhances the anti-tumor immune effect of tumor lysate-pulsed dendritic cell vaccine against glioblastoma. Oncol Transl Med. 2022;8(5):209–216.