Biochemical and Biophysical Research Communications 456 (2015) 513–518

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Exosome from chaperone-rich cell lysates-loaded dendritic cells produced by CELLine 1000 culture system exhibits potent immune activity

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ARTICLE INFO

Article history:
Received 20 November 2014
Available online 6 December 2014

Keywords:
Glioma
Dendritic cells (DCs)
DC-derived exosomes (DCex)
Chaperone-rich cell lysates (CRCL)

ABSTRACT

Dendritic cells (DCs) pulsed with exosomes can stimulate efficient cytotoxic T-lymphocyte responses and anti-tumor immunity. However, the quantity of DC-derived exosomes (DCex) obtained from various culture systems is very low, which is a significant practical issue hampering progress in this research area and needs to be addressed. Gliomas were particularly aggressive, with high morbidity and mortality, indicating that this is a form of incurable highly malignant tumor of the brain with poor prognosis. In the present study, we demonstrate that the CELLine 1000 culture system can dramatically increase the production of DCex. The morphology, phenotype and immune molecules of these DCex were found to be identical to those using traditional methods. Our researches supply a cost-effective, useful method for significantly increasing the quantity of exosomes. In addition, GL261 glioma cells were chosen to separate chaperone-rich cell lysates (CRCL). The results indicate that CRCL-GL261 cell lysates can trigger the most intense expression of immune molecules on DCex or DCs, which has important implications for the research into tumor treatment and diagnosis.

1. Introduction

Gliomas account for most malignant intracranial tumors with high morbidity, relapse rate and mortality [1,2]. Glioblastoma multiforme (GBM), as the most common primary brain tumor in adults, is an incurable highly malignant tumor with poor prognosis [3,4]. Following standard therapy, the average survival of GBM is 15–18 months [5]. Despite aggressive therapy with maximal safe surgical resection, radiation and chemotherapy, these tumors are invariably refractory to or become resistant to treatment [5]. One of the challenges in treating GBM is closely related to its localization within the naturally immunosuppressive central nervous system (CNS), which is anatomically-protected by a blood brain barrier and devoid of the traditional lymphatic structure [6].

Dendritic cells (DCs) are the major antigen-presenting and regulatory cells of the immune system, and play a central role in activation and regulation of the immune response [7–9]. Exosomes are 30–100 nm vesicles secreted from all types of cells, which has the potential to modulate intercellular communication [10,11]. The exosomal content is composed of different types of proteins, cytokines, growth factors, or nucleic acids [10]. Studies have demonstrated that DCs constitutively produce exosomes, which have recently been extensively characterized [11,12]. DC-derived exosomes (DCex) contain numerous plasma membrane and cytoplasmic DC components, incorporating a high number of major histocompatibility complex (MHC) and co-stimulatory molecules, and have been used for anti-tumor vaccines [13,14]. They can elicit very powerful immunogenic properties and are able to eradicate pre-established tumors in mice [15]. However, the quantity of DCex obtained from various culture systems is either very low, or requires scaling-up of cell culture systems requiring the need to handle high volumes of culture medium [16]. This is a significant practical issue hampering progress in this area.

A previous study showed that vaccination with DC loaded with tumor antigens can elicit tumor-specific immune responses potentially capable of killing cancer cells without inducing significant side-effects [17]. In the immunological arena, novel anti-cancer vaccines called chaperone-rich cell lysates (CRCL) have been found [18]. The primary characteristic of CRCL is the combination of

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http://dx.doi.org/10.1016/j.bbrc.2014.11.117
0006-291X/

chaperones, including HSP70 and HSP90 family members, and the endoplasmic reticulum (ER) chaperones glucose-regulated protein (GRP) 94/glycoprotein (gp) 96, and calreticulin. All of these chaperones have been utilized individually as anti-tumor vaccines [19,20]. Although mature dendritic cells (mDCs) pulsed with exosomes can stimulate more efficient cytotoxic T-lymphocyte responses and anti-tumor immunity than DCs or DCex, they are still less effective, showing very limited immune responses in clinical trials or only prophylactic immunity in animal models [14].

In the current study, we prepared 4 kinds of DCex ($DCex_{(t)}$, $DCex_{(1000)}$, $DCex_{(1000)}$-GL261 and $DCex_{(1000)}$-CRCL-GL261) and 4 kinds of DCs ($DCex_{(t)}$-DCs, $DCex_{(1000)}$-DCs, GL261-DCs and CRCL-GL261-DCs). We report that the CELLine 1000 culture system can dramatically increase the production of DCex, and that MHC-I, MHC-II, ICAM-I expression on $DCex_{(1000)}$ is equally effective as that on $DCex_{(t)}$. Both $DCex_{(t)}$-DCs and $DCex_{(1000)}$-DCs can significantly facilitate T cell proliferation with similar effectiveness than DCs. Meanwhile, CRCL-GL261 cell lysates can trigger the most intense MHC-I, MHC-II and ICAM-I expression as well as HSP70, HSP90 and TRP-2 on $DCex_{(1000)}$. Also, CRCL-GL261 can induce more intense antigen-presenting molecules and T-cell co-stimulatory molecules' expression on the surface of DCs, which is more effective than GL261 cell lysates.

2. Materials and methods

2.1. Animals

All experimental protocols were reviewed and approved by the institutional animal care and use committee in accordance with the Declaration of Helsinki. C57BL/6 female mice aged 8–10 weeks were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed under conventional conditions in accordance with institutional guidelines.

2.2. Antibodies

Rat anti-mouse ICAM-I, MHC-I antibody, rabbit anti-mouse MHC-II antibody, HRP-conjugated sheep anti-rat IgG antibody, HRP-conjugated sheep anti-rabbit IgG antibody, anti-β-actin antibody, FITC-labeled mouse anti-mouse MHC-I antibody, FITC-labeled rat anti-mouse MHC-II, CD80, CD86 antibody, isotype control antibodies, rat anti-mouse HSP90 antibody and rabbit anti-mouse HSP70, TRP-2 antibody were purchased from Abcam (Cambridge, MA).

2.3. Generation of BM-DCs by traditional methods

Bone marrow (BM) DCs were prepared as described previously with some modifications. Briefly, BM cells were collected and rinsed from mouse tibias and femurs. After removing red blood cells, BM cells were suspended and cultured in RPMI 1640 medium (Mediatec, Herndon, USA) containing 10% FBS (Fisher Scientific, Pittsburgh, USA) overnight. Bovine exosomes were removed from FBS by ultracentrifugation at 10,000×*g* for 18 h, followed by serial filtration (0.22 μm followed by 0.1 μm) using vacufilter units (Millipore, Billerica, USA). Non-adherent cells were then resuspended in fresh medium containing 10 ng/ml granulocyte–macrophage colony stimulating factor (GM-CSF; R&D Systems, Wiesbaden, Germany) and 10 ng/ml IL-4 (BD Pharmingen, San Diego, USA) in 15 ml Cell Culture Flasks. Cell medium was exchanged on days 3, 5, and 7, respectively. The supernatant was collected to produce $DCex_{(t)}$, which indicates exosomes were separated from culture solution of DCs by traditional methods. DCs were harvested and used in subsequent experiments [21].

2.4. Generation of BM-DCs by CELLine 1000 culture system

CELLine 1000 culture system was used to produce DCs. Briefly, 25 ml RPMI 1640 medium was added to medium compartment to infiltrate semipermeable membrane. Non-adherent BM cells (1×10^6/ml) in 15 ml RPMI 1640 medium containing 10% FBS, 10 ng/ml GM-CSF and 10 ng/ml IL-4 were added to the cell compartment. Then, RPMI 1640 medium (975 ml) was added to the medium compartment once more, and CELLine 1000 was placed in an incubator with 5% CO_2 at 37 °C for 7 days. The cell suspension was taken out from cell compartments, and half (7.5 ml) was given back. Also, 7.5 ml fresh RPMI 1640 with 10% FBS was supplemented. Medium in medium compartment was exchanged every three days. The culture solution in the medium and cell compartment was collected to produce $DCex_{(1000)}$, which indicated exosomes were separated from the culture solution of DCs by the CELLine 1000 culture system [16].

2.5. Preparation of DCex

Culture solution of DCs was collected and used to separate DCex as described previously [22]. Briefly, the culture solution was centrifuged at low speed (1000×*g*) for 10 min and filtered through 0.45 μm pore filters to remove cells and debris. Subsequently, cell/debris-free supernatant was concentrated in 100 kDa MWCO Centriplus centrifugal ultrafiltration (Millipore, Billerica, USA) by centrifugation at 1000×*g* for 30 min. DCex were separated from concentrated cell solution (10 ml) by ultracentrifugation at 100,000×*g* (Beckman L7-65 ultracentrifuge, 90-Ti rotor, Beckman Coulter) for 50 min. DCex were washed three times with PBS using 100 kDa MWCO Millipore Amicon (Millipore, Billerica, USA) and centrifugation at 1000×*g* for 30 min. Finally, DCex were resuspended in 10 ml PBS. Quantities of DCex were measured by BCA Kit (Thermo Scientific, Rockford, USA). Obtained DCex were filtered and degermed by 0.22 μm filter membrane, then stored at −80 °C [23].

2.6. Observation of DCex by TEM

Morphological characteristics of DCex were compared between $DCex_{(t)}$ and $DCex_{(1000)}$ by Transmission Electron Microscopy (TEM). Frozen exosomes were thawed on ice and resuspended in PBS (pH 7.4) containing 1% glutaraldehyde (Sigma, St. Louis, USA). A drop of 5 μl suspension was transferred onto a pioloform-coated copper grid and allowed to stand at room temperature for 5 min. Then, the grid was transferred to a drop of 50 μl double-distilled water for 2 min; this process was repeated seven times. The sample was stained by a drop of 5 μl 2% methyl cellulose (Sigma, St. Louis, USA) containing 2% uranylacetate (Sigma, St. Louis, USA) on ice for 10 min. Before viewing by TEM (Philips EM 208, FEI), excess fluid was removed by Whatman filter paper and allowed to air dry for 10 min[16].

2.7. Expression of ICAM-I, MHC-I, MHC-II on DCex by western blot

Total pre-enriched exosome sample ($DCex_{(t)}$ or $DCex_{(1000)}$) was diluted 1:1 in PBS, 5× RIPA lysis buffer (Sigma, St. Louis, USA) and proteinase inhibitor was added, and the sample was incubated at 4 °C for 15 min. The lysed exosome sample was mixed 1:1 with 2× Tris lysis buffer (pH 6.8). Samples were quantified by BCA. $DCex_{(t)}$ and $DCex_{(1000)}$ samples (50 μg/lane) were separated by SDS–PAGE on 10% polyacrylamide gels and transferred onto PVDF membranes (Sigma, St. Louis, USA). After blocking with 0.5% skimmed milk powder in PBS-Tween20 (Sigma, St. Louis, USA) for 1 h, the target proteins were probed with 1:1000 rat anti-mouse ICAM-I antibody or anti-β-actin antibody for 1 h at 4 °C.

After washing thrice (10 min/time) with PBS-Tween20, the membranes were incubated with HRP-conjugated sheep anti-rat IgG antibodies (1:4000 dilution by PBS) at room temperature for 30 min and washed again. Then, solutions A and B were mixed according to the instructions. The membranes were incubated with mixed solution for 1 min and exposed to X-ray. Images were observed after development and fixation. The same experiments were used to detect MHC-I and MHC-II [24].

2.8. The phenotype of DCex sensitized DCs by flowcytometry

During the exchange of culture medium, cultured DCs (day 5) were divided into two groups. Then, 10 μg/ml $DCex_{(t)}$ was added into 5×10^5 DCs for group A, while 10 μg/ml $DCex_{(1000)}$ was added into 5×10^5 DCs for group B. After incubation for 24 h, TNF-α (10 ng/ml, R&D Systems, Wiesbaden, Germany) was added to each group. $DCex_{(t)}$-sensitized DCs ($DCex_{(t)}$-DCs) and $DCex_{(1000)}$-sensitized DCs ($DCex_{(1000)}$-DCs) were obtained on day 8, respectively. The phenotype of $DCex_{(t)}$-DCs and $DCex_{(1000)}$-DCs was analyzed by flowcytometry (FCM). FITC-labeled anti-mouse MHC-I, MHC-II, CD80 or CD86 antibodies and isotype control antibodies of these molecules were used as reagents.

2.9. Mixed lymphocyte reaction (MLR)

Cultured DCs ($DCex_{(t)}$-DCs, $DCex_{(1000)}$-DCs or DCs) and autologous purified T cells were co-cultured overnight. Briefly, autologous purified T cells (responders) were separated from splenocytes of neonatal C57BL/6 mice. Here, 5×10^4 DCs (stimulators) had previously received irradiation at 30 grey. Then, stimulators (1×10^5 cells) and responders (1:1 ratio) were incubated in a 96-well plate with 5% CO_2 at 37 °C for 4 days. The plates were pulsed with [^{3}H] thymidine (1 μCi/well, Sigma, St. Louis, USA) for the final 18 h. Cells were then harvested using a Harvester 400 (Tomtec, Hamden, USA), and [^{3}H] thymidine incorporation was assessed using a 1450 MicroBeta counter (LKB Wallac, Ontario, Canada) [25]. Each treatment was repeated three times. The results were recorded by mean value per time (cpm ± SD).

2.10. Preparation of GL261 cell lysate

GL261 glioma cells purchased from ATCC were cultured in RPMI 1640 medium containing 10% heat-inactivated FBS. Then, GL261 glioma cells were frozen in liquid nitrogen for 10 min, melted at 4 °C and centrifuged at 12,000×*g* for 15 min. The supernatant was collected and quantified by BCA assay, then stored at −80 °C.

2.11. Preparation of chaperone-rich GL261 cell lysates (CRCL-GL261)

GL261 glioma cells (1×10^6) containing 1 ml RIPA and 10 μl PMSF (Sigma, St. Louis, USA) were centrifuged at the speed of 14,000 r/min at 4 °C for 30 min. The supernatant was collected and stored as the samples. Then, liquid-phase isoelectric focusing device was assembled. The sample and ampholyte were added into the device. The process of isoelectric focusing was carried out at 50 W of power in 4 °C circulating cooling water for 4 h. After that, sample was collected by vacuum pump and diluted by deionized water, then added to the device for second isoelectric focusing. The sample was collected and detected by BCA.

2.12. DCs were sensitized by GL261 or CRCL-GL261 cell lysates

GL261 and CRCL-GL261 cell lysates were used to sensitize DCs in the CELLine 1000 culture system. 50 μg/ml GL261 cell lysates or CRCL-GL261 cell lysates was added to day 7 cultured DCs in the cell compartment. After being co-cultured for 48 h, GL261-sensitized DCs (GL261-DCs), CRCL-GL261-sensitized DCs (CRCL-GL261-DCs) and culture medium were collected in turn. Also, GL261 cell lysate-sensitized $DCex_{(1000)}$ ($DCex_{(1000)}$-GL261) and CRCL-GL261 cell lysate-sensitized $DCex_{(1000)}$ ($DCex_{(1000)}$-CRCL-GL261) were obtained as described above.

2.13. Expression of immune molecules on $DCex_{(1000)}$ by immunoelectromicroscopy

Immunoelectromicroscopy was used to detect the expression of immune molecules on $DCex_{(1000)}$. For this, 10 μl $DCex_{(1000)}$ was mixed with rabbit anti-mouse HSP70 antibody diluted by different concentrations, and incubated at room temperature for 30 min, before being transferred for 4 °C incubation overnight. SPA collaurum was used to coat the mesh nickel grids. Briefly, 25 μl SPA collaurum (1:20 dilution) was dropped onto a hydrophobic membrane to form a droplet. The mesh nickel grid was placed on top of the droplet at room temperature for 20 min. Then, the antigen–antibody complexes prepared above were dropped onto the hydrophobic membrane to form droplets. The SPA-coated grid was floated on top of the droplet with antigen–antibody complexes, and incubated at room temperature for 2 h. The specimen was stained by phosphotungstic acid (20 ml/L, pH 6.8). An SPA-coated grid floated on top of the droplet with PBS was used as control. The same experiments were carried out to detect the expression of HSP90, and TRP-2 on $DCex_{(1000)}$. The results were observed by TEM. Also, the number of positive $DCex_{(1000)}$ was counted [26]. The expression of ICAM-I, MHC-I and MHC-II in $DCex_{(1000)}$was detected by western blot. The phenotype of GL261-DCs and CRCL-GL261-DCs was observed by flowcytometry.

3. Results

3.1. Morphological characteristic of DCex

To define purity and quality, we examined DCex morphology and quantity using TEM. TEM showed that purified DCex were a relatively homogenous population of micro-vesicles with a typical diameter of 30–100 nm and an oval-biconcave shape (Fig. 1). DCex were undamaged during purification and purified DCex were not contaminated with the micro-particles of cellular organelles or membranes. In addition, the number of $DCex_{(1000)}$ was much higher than that of $DCex_{(t)}$. This result indicated that the application of CELLine culture system can dramatically increase the production of DCex.

3.2. Expression of MHC-I, MHC-II, ICAM-I on DCex

Previous studies have demonstrated that DCex contain cytoplasmic DC components and numerous plasma membranes. Several plasma membrane molecules that are relevant for the immune functions of DCs are also expressed on the DCex bounding membrane. These include Class I and II MHC molecules and intercellular adhesion molecule-1 (ICAM-I) [12,27]. According to experiments we described in methods, there are 4 kinds of DCex were obtained: $DCex_{(t)}$, $DCex_{(1000)}$, $DCex_{(1000)}$-GL261 and $DCex_{(1000)}$-CRCL-GL261, respectively. In order to evaluate and compare the expression of MHC-I, MHC-II and ICAM-I on DCex, western blot was carried out and the integrated optical density (IOD) of the bands for target proteins was analyzed. As shown in Fig. 2A and B, the expression of these three molecules is similar between $DCex_{(t)}$ and $DCex_{(1000)}$ ($P > 0.05$), which suggests that the production of $DCex_{(1000)}$ is equally as effective as $DCex_{(t)}$. In addition, CRCL-GL261 can trigger the most intense MHC-I, MHC-II and ICAM-I ($P < 0.01$, $DCex_{(1000)}$-CRCL-GL261 vs. $DCex_{(1000)}$; $P < 0.05$,

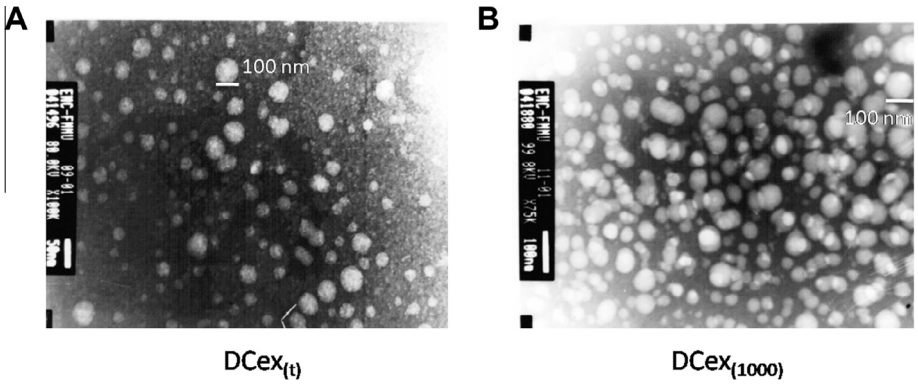

Fig. 1. The morphological characteristic of purified DCex by TEM. DCs were generated from bone marrow cells in the presence of GM-csF/IL4/Lps by traditional and CELLine 1000 culture systems, respectively. DCex were isolated from conditioned media of these DC cultures using fractionated centrifugation and filtration. (A) $DCex_{(t)}$. (B) $DCex_{(1000)}$. The length of the white bar in corresponding images represents 100 nm.

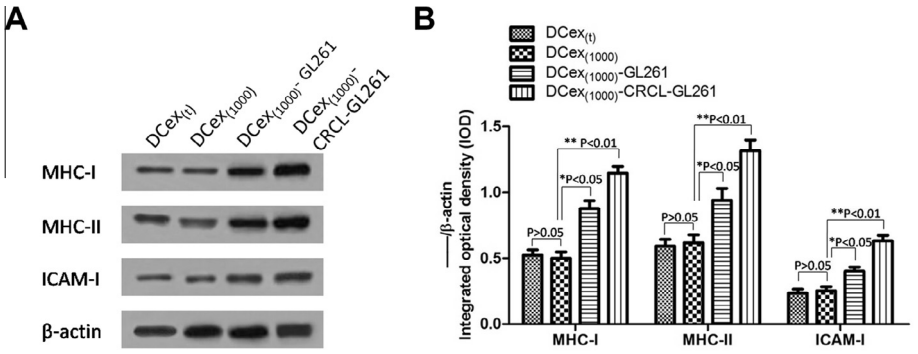

Fig. 2. Expression of MHC-I, MHC-II and ICAM-I on DCex was detected by western blot. $DCex_{(t)}$, $DCex_{(1000)}$, $DCex_{(1000)}$-GL261 and $DCex_{(1000)}$-CRCL-GL261 were obtained as described in methods. The bands for targeted proteins of WB repeated three times were analyzed by Gel-Pro analyzer 4.0 software. (A) Expression of MHC-I, MHC-II and ICAM-I by WB. (B) Integrated optical density (IOD) for MHC-I, MHC-II and ICAM-I. Data were normalized based on the β-actin levels. $^{*}P < 0.05$ and $^{**}P < 0.01$ by student's test when compared with $DCex_{(1000)}$.

$DCex_{(1000)}$-GL261 vs. $DCex_{(1000)}$) expression on $DCex_{(1000)}$. These results suggested that CRCL may be the best vaccine to load with DCex, which can amplify the potent immunocompetence of DCs.

3.3. Effect of DCex on T cell proliferation by MLR

The effects of $DCex_{(t)}$-DCs and $DCex_{(1000)}$-DCs on T cell proliferation were evaluated by MLR. DCs, $DCex_{(t)}$-DCs or $DCex_{(1000)}$-DCs were used as stimulators, while autologous purified T cells as responders. MLR suggested that both $DCex_{(t)}$-DCs and $DCex_{(1000)}$-DCs can significantly facilitate T cell proliferation with similar effectiveness (Fig. 3C) when compared with DCs alone ($^{*}P < 0.05$). This result suggests that DCex can trigger more intense T cell proliferation. According to the above results, $DCex_{(t)}$ can be instead by $DCex_{(1000)}$ in a way. The CELLine 1000 culture system is more effective than traditional methods.

3.4. Expression of immune molecules on $DCex_{(1000)}$-GL261 and $DCex_{(1000)}$-CRCL-GL261

In order to explore whether GL261 or CRCL-GL261 cell lysates can influence the expression of immune molecules on $DCex_{(1000)}$, we constructed GL261-$DCex_{(1000)}$ and CRCL-GL261-$DCex_{(1000)}$, which were isolated from GL261-DCs and CRCL-GL261-DCs, respectively. Also, immunoelectromicroscopy was used to detect the expression of immune molecules including HSP70, HSP90 and TRP-2. As shown in Fig. 4A and B, the number of positive cells for each target protein in $DCex_{(1000)}$-CRCL-GL261 is higher than that in $DCex_{(1000)}$-GL261. This result indicated that CRCL-GL261 cell lysates can induce the more intense expression of immune molecules on $DCex_{(1000)}$, which is more efficacious than GL261 cell lysates.

3.5. Phenotype of DCs

DCs have been characterized as professional antigen-presenting cells (APCs), the main function of which is to cross-prime effector T cells against specific proteins or glycolipid antigens [28]. The surface of DCs is enriched in antigen-presenting molecules such as MHC-I and MHC-II, and T-cell co-stimulatory molecules such as CD80 and CD86, which are relevant for the immune functions. In the current study, $DCex_{(t)}$-, $DCex_{(1000)}$-, GL261- and CRCL-GL261-sensitized DCs were obtained. Flow cytometric analysis of DCs demonstrated that MHC-I, MHC-II, CD80, and CD86 were expressed on all of these DCs. There was no significant difference of their expression between $DCex_{(t)}$- and $DCex_{(1000)}$-sensitized DCs (Fig. 4A), while CRCL-GL261-sensitized DCs expressed these markers at higher levels than GL261-sensitized DCs (Fig. 4B). These results indicated that $DCex_{(1000)}$ has the same ability as $DCex_{(t)}$ to sensitize DCs, and demonstrated the ability of the CELLine 1000 culture system. In addition, CRCL-GL261 can induce the more intense expression of antigen-presenting molecules and T-cell co-stimulatory molecules on the surface of DCs than GL261.

4. Discussion

This study focused our interest on the DC-derived exosomes (DCex), which have recently been extensively researched and reported [29,30]. Later studies indicated that mDCs pulsed with

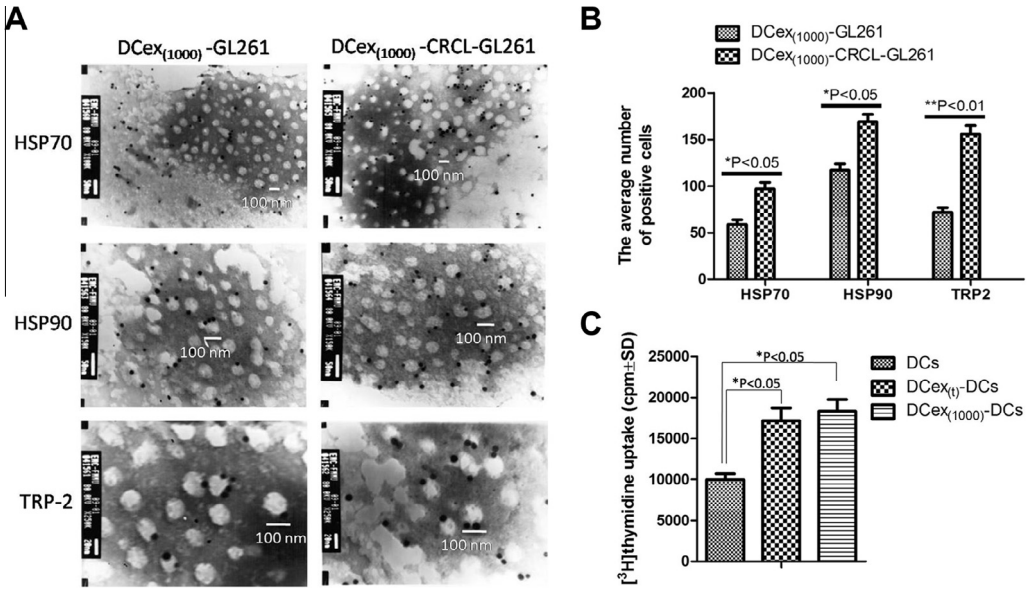

Fig. 3. Detection of HSP70, HSP90 and TRP-2 on $DCex_{(1000)}$. $DCex_{(1000)}$-GL261 and $DCex_{(1000)}$-CRCL-GL261 were obtained as described above. (A) Expression of HSP70, HSP90 and TRP-2 on $DCex_{(1000)}$ by TEM. (B) The number of HSP70, HSP90 or TRP-2 positive cells. $^*P < 0.05$ by student's test when compared with $DCex_{(1000)}$-GL261. (C) T cell proliferation was evaluated by MLR. $DCex_{(t)}$-DCs and $DCex_{(1000)}$-DCs were used as stimulators, respectively. $^*P < 0.05$ by student's test when compared with DCs.

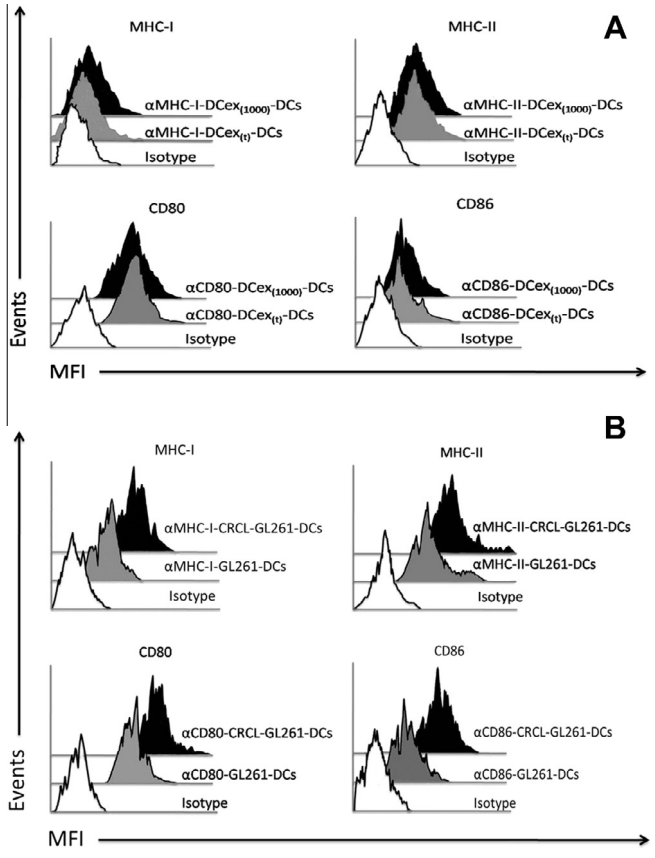

Fig. 4. Phenotype of $DCex_{(t)}$-, $DCex_{(1000)}$-, GL261- and CRCL-GL261-sensitized DCs. Surface markers on these DCs were analyzed by flow cytometry. DCs were stained with fluorochrome-conjugated antibodies specific for the DC markers class I MHC (αMHC-I), class II MHC (αMHC-II), CD80 (αCD80) and CD86 (αCD86), as well as isotype control antibodies (Isotype). (A) Phenotype of $DCex_{(t)}$- and $DCex_{(1000)}$-sensitized DCs. (B) Phenotype of GL261- and CRCL-GL261-sensitized DCs. Data are mean fluorescence intensity (MFI) of a representative experiment of 3 independent experiments performed.

exosomes can stimulate efficient cytotoxic T-lymphocyte responses and anti-tumor immunity [14]. The purification of exosomes requires the maintenance of cells at high density to obtain sufficient accumulation of exosomes in culture medium. While high density cultures can be achieved with cells in suspension, this remains difficult with adherent cells, resulting in a low quantity of exosomes for subsequent study [16].

In this study, DCex were harvested from the DC culture supernatants by ultracentrifugation. Our data showed that the CELLine 1000 culture system, which is more effective, can dramatically increase the production of DCex. The morphology, phenotype and immune molecules of $DCex_{(1000)}$ were compared with $DCex_{(t)}$, and found to be identical in this respects. Also, $DCex_{(1000)}$ has the same ability and availability as $DCex_{(t)}$ to sensitize DCs. Hence, we conclude that traditional methods can be completely replaced by the CELLine 1000 culture system, and the application of this system to product DCex will facilitate the progress in related research areas. Our research supplies a cost-effective, lower labor, useful method for significantly increasing the quantity of DCex without any detrimental effects.

A previous study demonstrated that tumor-derived chaperone-rich cell lysates (CRCL) carry antigenic peptides. Also, DCs can uptake CRCL and cross-present the chaperoned peptides to T cells, thus inducing protective immune responses against a diverse range of murine tumor types in different genetic backgrounds [18]. For example, Zeng et al. found that CRCLs are potent immunologic agents against a variety of murine tumors, including 12B1. They demonstrate that the combination of imatinib with DCs loaded with 12B1-derived CRCL yields high activation of anti-12B1-specific T cells and potent anti-tumor activity [31]. The above findings sparked our interest in the role of DCex-CRCL in immune responses against tumors.

In this article, GL261 glioma cells were chosen as tumor cells to separate CRCL-GL261 cell lysates. The results indicate that CRCL-GL261 cell lysates can trigger the most intense MHC-I, MHC-II and ICAM-I expression as well as HSP70, HSP90 and TRP-2 on $DCex_{(1000)}$. In addition, CRCL-GL261 can induce more intense and efficacious antigen-presenting molecules and T-cell co-stimulatory molecules expression on the surface of DCs than GL261 cell lysates.

This finding first reports the effect of CRCL-GL261 on the expression of immune molecules on DCex or DCs, which has important implications for the research into tumor treatment and diagnosis. Also, the resistance of gliomas by $DCex_{(1000)}$-CRCL-GL261 will be explored in further research.

Acknowledgments

This study was supported by grants from National Natural Science Foundation of China (No. 81201983), Special Research Foundation of Personnel Training of the Second Affiliated Hospital of Xi'an Jiaotong University Medical School (New Talents of Technology) (No. RC(XM)201201), and Free Exploration and Independent Innovation Projects of Xi'an Jiaotong University (No. xjj2012058).

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