Umbilical cord blood-derived dendritic cells loaded with BGC823 tumor antigens and DC-derived exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumor immunity *in vitro* and *in vivo*

SHASHA GUAN, QIANRU LI, PINGPING LIU, XIAOYAN XUAN, YING DU

Department of Immunology and Microbiology, Basic Medical College, Zhengzhou University, PR China

Abstract

Background: *Umbilical cord blood (UCB) is a rich source of hematopoietic stem cells and from which a significant number of dendritic cells (DCs) can be produced. But the therapeutic role of DCs and exosomes (EXO) generated from DCs is not fully elucidated.*

Material and methods: *The UCB-derived DCs were loaded with tumor antigens generated from BGC823 cell line. Exosomes were derived from these DCs by ultracentrifugation. Dendritic cells and DCex were evaluated by light microscope, transmission electron microscope (TEM), flow cytometry, and western blot assay. The therapeutic role of DCs and EXO generated from DCs were then detected in vitro and in vivo.*

Results: *Dendritic cells isolated from umbilical cord blood after loading with tumor antigens generated from BGC823 cell line could express high levels of protein molecules: MHC-I, MHC-II, CD34, CD40, CD80, CD86, CD11c and CD54 and mediate a stronger promotion of T cells proliferation. And, they could also enhance the cytotoxicity effects of the generated CTL in vitro and in vivo. Exosomes isolated from these DCs were 40-90-nm round particles with a complete membrane structure and could also expressed molecules similar to DCs. Exosomes could stimulate T cell proliferation, produce effective cytotoxicity and induce more efficient in vivo antitumor immunity.*

Conclusions: *These results suggested that tumor antigens loaded DCs derived from unrelated umbilical cord blood or DCex can induce tumor specific cytotoxicity and this may represent a novel immunotherapy for tumors. Because of their advantage of stable, easy to store, DCex have a more brilliant prospects in the tumor immunity.*

Additional information:

We reported that exosomes derived from umbilical cord blood dendritic cell (UBDC), similar to DCs, can trigger activation of T cells significantly. These data demonstrate that DC-derived exosomes (DCex) can mediate essential adaptive immune functions.

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Key words: *antitumor immunity, BGC823 cell line, dendritic cells, exosomes.*

Dendritic cells (DCs) play an important role in the immune system. They can cause a wide range response that span from the development of self-tolerance to the elicitation of potent cellular and humoral immunity [1]. Dendritic cells are in an attempt to be used as therapeutic anticancer vaccines after loaded with tumor-associated antigens (TAAs) [2, 3]. And a large array of cancer vaccination strategies based on DCs have been developed. And exosomes are the most popular ones [4, 5].

Dendritic cell-derived exosomes (DCex) which are released in tumor cells, leukocytes, blood platelets, mast cells, enterocytes and red blood cells etc. can activate adaptive immune responses because they are not only highly enriched in MHC Class II molecules, but also can express high levels of co-stimulatory molecules including CD40, CD80 and CD86 [6]. Since 1998, Zitvogel *et al.* [7] have proposed the use of cell-free vaccines in tumors, showing that DC-derived exosomes promote a T cell-de-

Correspondence: Xiaoyan Xuan, Ying Du, 100 Kexue Road, Department of Immunology and Microbiology, Basic Medical College, Zhengzhou University, Zhengzhou, Henan, China, 450052, e-mail: xuanxiaoyan@163.com, duying@zzu.edu.cn

pendent antitumor immune response *in vivo*. In line with this, Cho *et al.* [8] reported the use of exosomes engineered to express a specific tumor antigens to generate an immune response against tumors.

The vaccine approach in cancer from exosomes cannot be popularized because of the fact that exosomes must be prepared locally using the treated patient's DCs. Thus, it is a must to find a substitute for the precursor cells of DCs. In 2004, Matsuyosh *et al.* [9] reported that umbilical cord blood dendritic cell (UBDC) can be used as a substitute in tumor immunotherapy. Up to now, there has been no proof of it. Here we reported that exosomes derived from UBDC, similar to DCs, can trigger activation of T cells significantly. These data demonstrate that DCex can mediate essential adaptive immune functions.

Material and methods

Reagents and antibodies

Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF), recombinant human stem-cell factor (rhSCF), recombinant human interleukin-4 (rhIL-4), and rhIL-2 were purchased from PeproTech (USA). Mouse mAb anti-human MHC-I, MHC-II, CD34, CD40, CD80, CD86, CD11c, CD54 and FITC-conjugated sheep anti-mouse immunoglobulin (Ig) G were purchased from Ancell (USA). Trizol reagent and Lipofectamine™ reagent were purchased from Invitrogen (USA).

Cell lines and cell culture

Tumor cell line BGC823 (a kind gift from the cell center of the Chinese Academy of Medical Sciences, Shang Hai) were grown in RPMI 1640 with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2. Fetal bovine serum contains endogenous exosomes, so the cell culture medium was replaced with serum deficient medium 24 h before exosomes collection.

Animals

Twenty male 6- to 8-week-old BALB/c nu/nu mice, with an average weight of 18-22 g, were purchased from the Chinese Academy of Sciences. Animals were treated and cared about ethically and humanely and all animal studies were conducted under approved institutional protocols.

Induction of dendritic cells from human umbilical cord blood

Collection of human umbilical cord blood (UCB) samples was approved under the First Affiliated Hospital of Zhengzhou University guidelines. Umbilical cord blood samples were obtained from normal full-term deliveries after informed consent was obtained from them. The Ficoll-Hypaque density gradient centrifugation method was used to isolated the monocytes from UCB samples. Cell density was adjusted to 2×10^8/l using RPMI 1640 supplemented with 15% heat-inactivated FCS, put in the culture flask and incubated in 37 50 ml/l CO_2 for 2 h. The nonadherent cells were get rid of and the adherent cells were collected and replated at 2×10^8 cells/l in new 6-well petri dishes. The medium was supplemented with 50 ng/l human recombinant SCF, 50 ng/ml rhGM-CSF, and 10 ng/ml rhIL-4. The monocyte cell culture was fed every 2 days by gentle replacement of 50% of the medium with fresh medium. After incubation for 9 days, the morphology of monocytes derived immature DC (imDC) were observed through light microscope and the expression of DC surface markers were determined by flow cytometry assay.

BGC823 cell total RNA or lytic-Ag loading of cultured dendritic cells

Total RNA was extracted from BGC823 cells using Trizol reagent according to the manufacturer's instructions. Tumor cell lytic-Ag was generated by four rapid freeze-thaw cycles as described. RNA loading of DCs was performed by simple co-incubation of DCs with lipofectamine reagent while lytic-Ag loading of DCs was performed without any transfection reagent. In brief, imDC generated on day 8 were washed twice in PBS, counted, and resuspended in AIM-V medium supplemented with rhSCF, rhGM-CSF and rhIL-4. Then, tumor cells RNA or lysates were added (20 µg RNA or 100 µg lysates/ 5×10^5 imDCs in 1 ml medium) and incubated with DCs for 3 hours in a humidified incubator at 37°C/5% CO_2. After combination, fresh complete medium (including rhSCF, rhGM-CSF, rhIL-4 for DCs) was added to each well. After incubation for 2 days, the morphology of DC loaded with BGC823 cell total RNA or Lytic-Ag was observed through light microscope and the expression of DC surface markers was determined by flow cytometry assay.

Flow cytometry analysis of dendritic cells

For the flow cytometry examination of imDC or DCs loaded with TAAs surface markers, the following antibodies were used: mouse anti-human mAb MHC-I, MHC-II, CD34, CD40, CD80, CD86, CD11c and CD54 (diluted by 1 : 100). Supernatants were discarded. Dendritic cells were incubated with those antibodies mentioned above for 30 min at room temperature, then washed 2 times with PBS. The supernatant was removed and 100 µl of FITC-conjugated sheep anti-mouse IgG was added. Green fluorescence at a wavelength of 488 nm was tested and recoded. The data were analyzed with Expo32ADC.

Exosomes isolation

Dendritic cells loaded with TAAs were cultured in serum-free medium to eliminate bovine serum exosome contamination. 200 ml of serum-free medium of DCs was

centrifuged at 2000 × *g*, 4°C for 10 min to remove dead cells. And then the supernatant was centrifuged again at 10,000 × *g*, 4°C for 30 min to remove protein aggregates and cell debris. Samples were transferred to ultracentrifuge tubes and were centrifuged at 100,000 × *g*, 4°C for 2 h to pellet the exosome fraction. The final pellet, representing the purified exosome fraction, was resuspended in a small volume of PBS and stored at –80°C, exosome protein amounts were measured using the BCA protein assay kit.

Exosome preparation for transmission electron microscopy

Exosomes were washed 2 times with PBS, and fixed in 3% formaldehyde and 0.1% glutaraldehyde (EM grade) for 10 min at 37°C. Then exosomes were loaded on Formvar/carbon-coated grids, negative stained and observed with a transmission electron microscope.

One-dimensional SDS-PAGE analysis and western blot analysis of exosomes

Exosome proteins (20-40 mg) separated by SDS-PAGE gel electrophoresis were then transferred onto a nitrocellulose membrane. Blots were blocked at room temperature for 1 h and then incubated with primary antibody: mouse monoclonal anti-MHC II, anti-CD40, anti-CD80, anti-CD86 and anti-CD54 at 4°C overnight. After washing, the blots were incubated with FITC-conjugated sheep anti-mouse IgG (1 : 2000) for 1 h. Protein bands were detected (the colored membranes) with the enhanced chemiluminescence (ECL) system and exposed to X-ray film. Experiments were performed three times for each experimental condition.

T-cell proliferation assays

Monocytes were isolated from cord blood, the adherent cells were taken out to induce to DCs. The nonadherent cells were washed 2 times with Hank's buffer and the density was adjusted to 1×10^{11}/l with RPMI 1640 supplemented with heat inactivated FCS (10% v/v). Then, they were filtered through a nylon mesh to separate T cells and the density was adjusted to 2×10^9/l. Cells were analyzed by multicolor flow cytometry. The result showed that the purity coefficient of CD3+ T cells could be 92%. In a mixed lymphocyte reaction (MLR) experiment, T-cells (2×10^9) were incubated with DCs loaded with or without TAAs in a final volume of 200 μl per well in 96-well plate, at a T-cells to DCs ratio of 100 : 1, 50 : 1, 10 : 1, respectively. On the other hand, T-cells (2×10^9) from human cord blood were suspended and plated (100,000 per well) in 96-well plate. Exosomes of 1 μg, 2 μg, 5 μg, 10 μg, 20 μg were added to the wells of the experimental group, and identical volume cell culture fluid was added to the wells of the control group. The plates were gently shaken for 1 h at room temperature, to ensure exosome-T cell interaction. After 3 days' culture, MTT methods were used to determine the stimulate index (SI). Briefly, MTT reagent was added to each well at 5 mg/ml in 20 μl and cells were incubated for another 4 h. Dimethyl sulfoxide (DMSO) was then added and the final products were quantified by spectrophotometer at 490 nm for OD values. Stimulate index (SI) was calculated as the following equation: SI = experimental/control. "Control" means the OD value obtained from the wells containing T cells alone, and "experimental" means the OD value obtained from the wells containing both T cells and DC loaded with or without TAAs or exosomes.

MTT cytotoxicity assay

Tumoricidal activity of exosomes or DCs loaded with TAAs was tested using a modified MTT cytotoxicity assay. BGC823 cells were suspended in complete cell culture media and plated (2,500 per well) in a flat bottom 96-well plate. T cells cultivated together with DCs loaded with TAAs (ratio at 10 : 1) or with exosomes for 3 days were harvested and were added into the well, at a T-cells to BGC823 cells ratio of 10 : 1, 20 : 1, 50 : 1. After 2 days' culture, percentages of cytotoxicity were calculated using MTT. Percentages of cytotoxicity were calculated using the following formula [(control – experimental)/control] × 100.

In vivo assay

Tumorigenesis assays were performed in 6-8-week-old athymic BALB/c (nu/nu) male mice. In all experiments, groups of four mice were injected subcutaneously with $1 ′ 10^7$/ml BGC823 cells suspended 2n 100-μl of FBS-free 1640. After the tumors grew to 100-200 mm³, mice were treated peritumorally with either: i) 50 μg exosome + T cells ($2 ′ 10^6$ cells), ii) DCs transfected with BGC823 cells total RNA cell ($2 ′ 10^5$) + T cell, iii) DCs pulsing with BGC823 cells lytic-Ag + T cell, iv) imDCs + T cell, or v) normal sodium by a subcutaneous injection. At the same time, the mice were injected subcutaneously with IL-2 (1000 u) for 5 days. The mice were retreated after day 11. Tumor growth was monitored and recorded weekly. Tumor size was measured with an electronic caliper and size-volume was estimated using the formulae a × b2 × (π/6) = V (mm³) (a = major diameter; b = minor diameter and V = volume). Treatment lasted for 60 days. At day 61, mice were sacrificed and necropsy performed to evaluate the tumor weight.

Statistical analysis

The database was set up with the SPSS 17.0 software package for analysis. Data were presented as mean ± SD. The means of multiple groups were compared with one-way analysis of variance (ANOVA). Statistical comparison was also performed with two-tailed *t* test when appropriate. *P* < 0.05 was considered statistically significant.

Results

Phenotype of immature dendritic cell and matured dendritic cell

The precursor cell of UCB-DC separated from umbilical cord blood monocytes were cultured and induced in complete RPMI 1640 medium supplemented with a given dose of rhGM-CSF, rhIL-4 and rhSCF. Adherent aggregates were visible on day 3. After 9 days, the number of cells increased about 10.69 ±3.52-fold compared with the number of cells before culture. And the DCs were ridgy in shape with a relative smooth membrane surface under a light microscope (Fig. 1A), demonstrating that they are mostly in immature status. To assess more specifically the phenotype of DC, several mAbs directed against surface markers were used. The results presented in Figure 1B showed that the generated DCs were MHC class I+, MHC class II+, CD3+, CD40+, CD80+, CD54+, CD11c+, and CD86+, which all have been reported to be expressed by DCs but all at very low levels. Next, DC phenotype changes were detected by the light microscope and flow cytometry to analyze the effects of BGC823 total RNA or tumor antigens on DC maturation. As shown in Figure 1C, DCs changed greatly, and some of them became significantly larger in size with rough surface, richer ruffles on the cell membrane, and bigger, longer protrusions. The formation of roughness, and protrusion on the cell membrane are considered to be associated with maturation of DC. These results suggest that there exist obviously morphologic characteristics of mature DC. Generally speaking, it is considered that the morphologic change is the foundation of the function of DC. The results of flow cytometry showed that both BGC823 total RNA and tumor antigens pulsing enhanced CD80, CD86, MHC and CD54 expression significantly, especially for those UBDC transfected with BGC823 total RNA. These results indicated that BGC823 total RNA or tumor antigens pulsing could promote costimulatory molecules and maturation marker expression of mature DCs (Fig. 1C).

Phenotype of exosomes

To verify the purity and quality of exosomes secreted by DC loaded with TAAs, we performed morphologically and phenotypically characterized analysis then by transmission electron microscopy (TEM) and flow cytometry, respectively. Transmission electron microscopy showed that isolated exosomes were 40-90-nm round particles with a complete membrane structure. The arrow points to the characteristic cup-shaped morphology and the sizing bar indicates that the vesicles have the characteristic diameter range of 40-90-nm (Fig. 2A). Western blot analysis of exosomes demonstrated that the classical DC surface markers MHC-II, CD40, CD80, CD86 and CD54 were also expressed on exosomes from DC (Fig. 2B).

Stimulation of T-cell proliferation

To assess the functional consequences of BGC823 tumor whole antigen-pulsed DCs, we examined the T cell stimulatory index of tumor total RNA transfected or lytic-Ag pulsed DCs MTT. As shown in Figure 3A, the stimulatory ability of imDCs and control was much lower, in contrast, both tumor RNA transfected or lytic-Ag pulsed DCs have potent stimulatory effects on T cells proliferation ($p < 0.05$). Interestingly, the stimulatory ability of DCs transfected with BGC823 cells total RNA was higher than DCs pulsing with BGC823 cells lytic-Ag perhaps because loading total RNA decreases the probability of immune escape via polyclonal activation of T cells against varied range of tumor-specific antigens and minimizes the consequences of antigen loss in mutant tumor cell clones, and the total RNA loaded DCs could efficiently present functional antigenic peptides and co-stimulatory signals to T cells.

To investigate the effects of DEex on T cells *in vitro*, we incubated T cells in the presence of exosomes produced from tumor RNA transfected or lytic-Ag pulsed together for 72 h in different concentration: 1 μg, 2 μg, 5 μg, 10 μg, 20 μg. The T cells proliferation ability was detected by MTT. The results showed that DEex produced from tumor RNA transfected or lytic-Ag pulsed DCs could stimulate T cells proliferation efficiently from 2 μg compared to that in control or 1 μg ($p < 0.05$) and it was dose-dependent, as shown in Figure 3B. These data confirmed that umbilical cord blood DC-derived exosomes could directly activate T cells *in vitro*.

Cytolytic activity of the T cells against BGC823 cell lines *in vitro*

First, we detected cytotoxic activity of T cells induced by DC loaded with RNA or lytic-Ag generated from the BGC823 cell line. As demonstrated in Figure 4A, both tumor total RNA transfected or lytic-Ag pulsed DCs were capable of stimulating T cells into CTLs that recognized and lysed BGC823 cell lines. At the same time, loading DCs with RNA could induce the better lytic activity than DCs loaded with lytic-Ag. It also indicated that loading DC with RNA might stimulate higher cytolytic activity of the T cells against BGC823 cells compared to that in controls ($p < 0.05$). While immature DCs have no ability of stimulating CTL specific cytotoxicity.

Next, we detected cytotoxic activity of T cells induced by DCex. We checked the T cells incubated with 2 μg DCex cytolytic ability by using MTT assay. As shown in Figure 4B, DCex derived from DCs loaded with RNA or lytic-Ag from the BGC823 cell line could stimulate CTL specific cytotoxicity efficiently compared to that in controls ($p < 0.05$). Cytotoxicity against DCex derived from DCs loaded with total tumor RNA was significantly higher than the corresponding value for DCex derived from DCs loaded with lytic-Ag ($p < 0.05$).
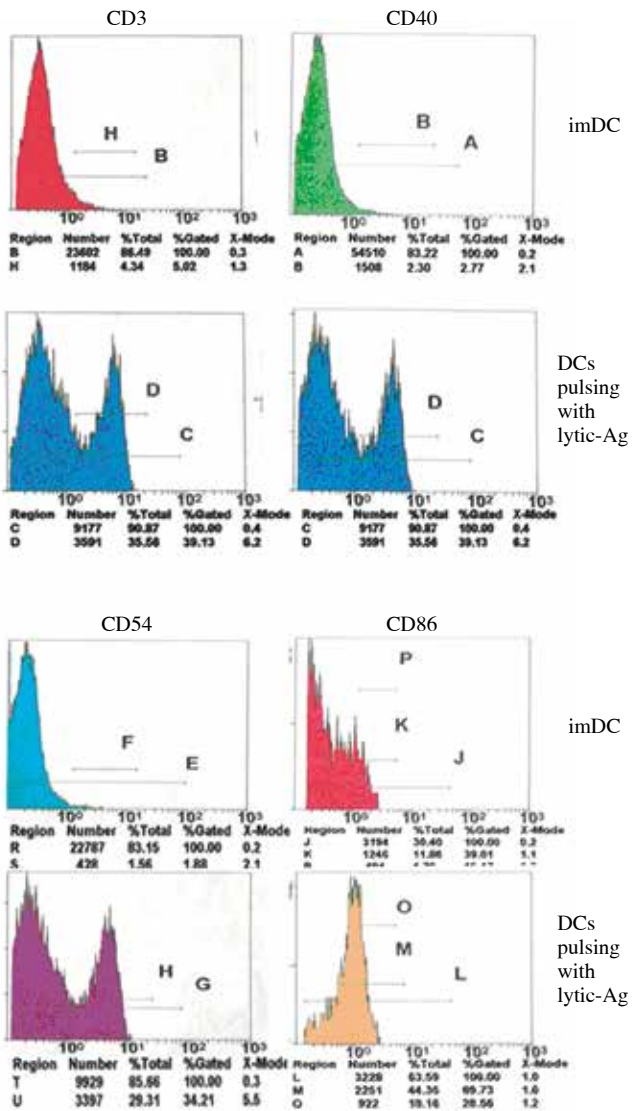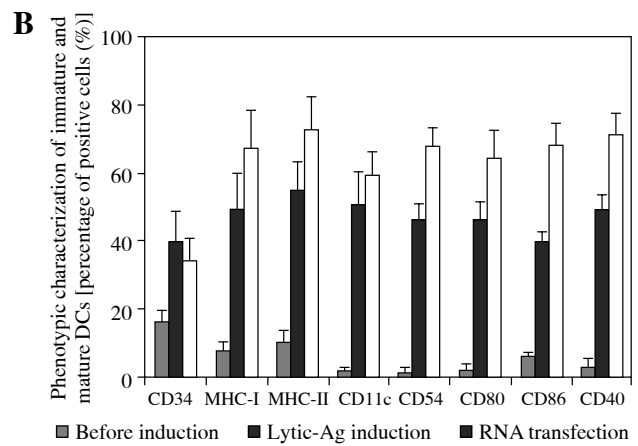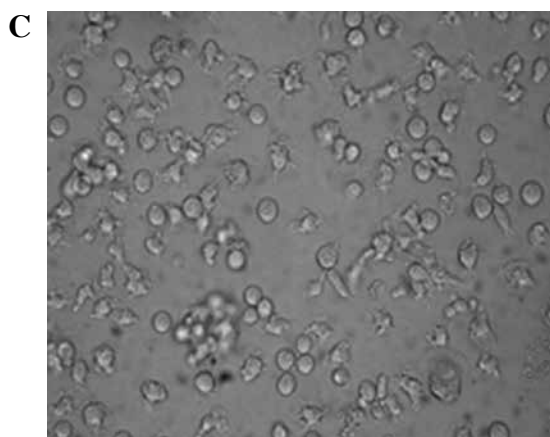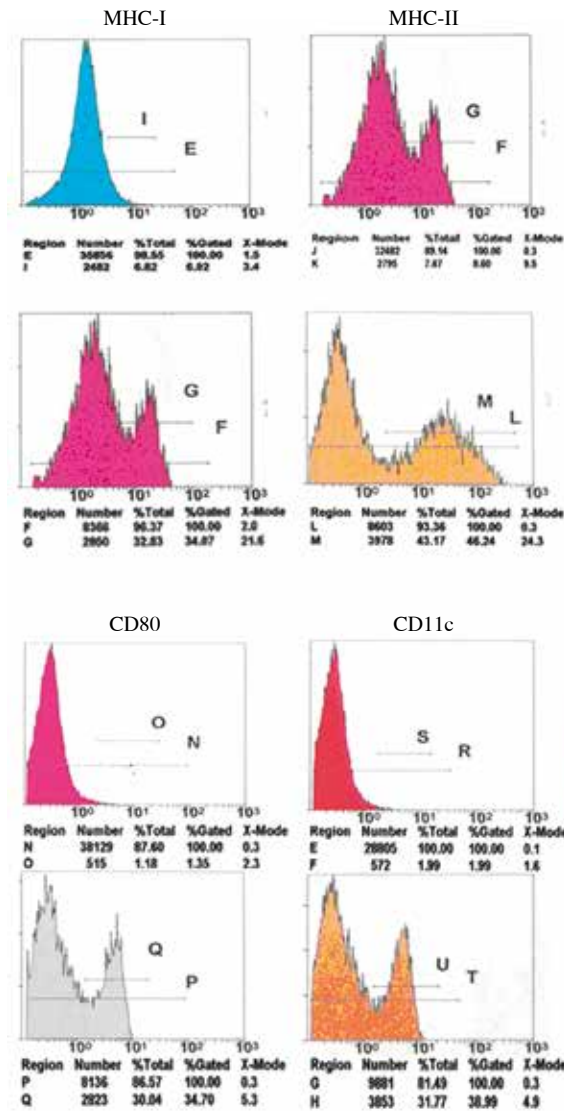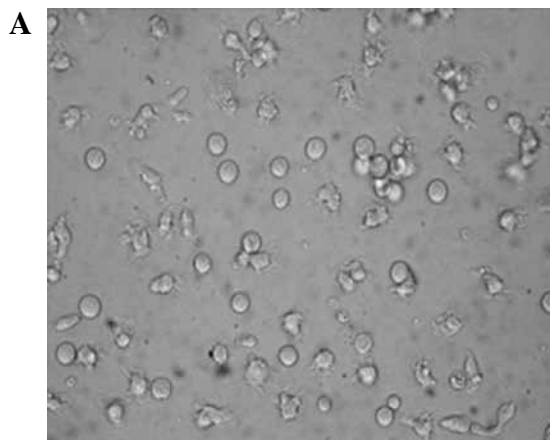
Fig. 1. Phenotype of imDC and matured DC
Transmission electron microscopy and flow cytometry assay were used to evaluate the phenotype of imDCs generated from UCB after 6 days of culture in the presence of rhSCF, rhGM-CSF and rhIL-4, and mature DCs treated by BGC823 TAAs for 2 days. **A)** The morphology of the imDCs was observed under a light microscope (magnification 200×). **B)** The expression of several molecules (MHC-I, MHC-II, CD3, CD40, CD80, CD54, CD11c, and CD86) was significantly higher in the DCs loaded with tumor antigens than in the immature DCs, $p < 0.01$. Furthermore, the expression of molecules on the DCs transfected with BGC823 cells total RNA were higher than of those on the DCs loaded with lytic-Ag of BGC823 cells, $p < 0.05$. Data in the flow cytometry pictures were from one representative experiment of four independent experiments. **C)** The morphology of the mature DCs was observed under a light microscope (magnification 200×)
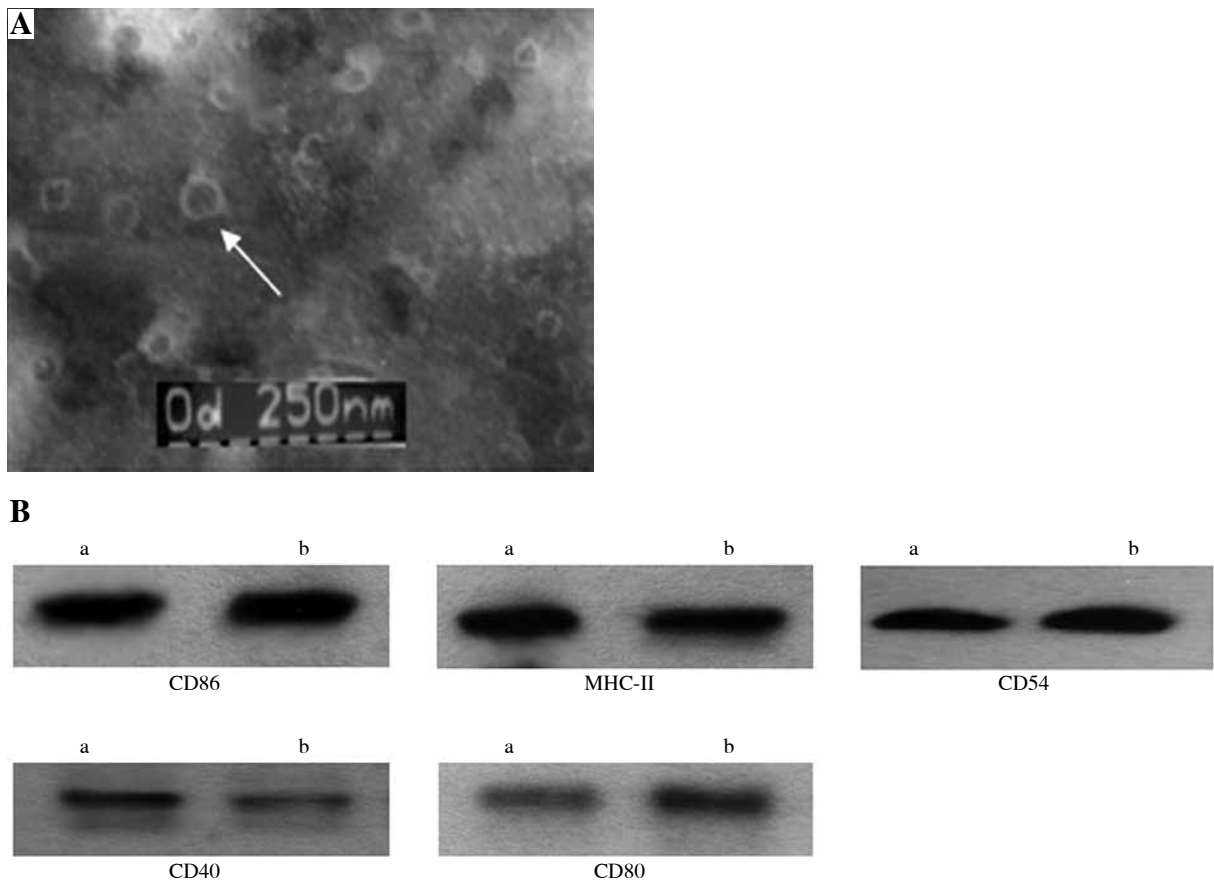
Fig. 2. Phenotype of exosomes
Exosomes were isolated from conditioned media of tumor antigens loaded DC cultures using fractionated centrifugation and filtrations. Transmission electron microscopy, and western blot assay were used to evaluate the phenotype of exosomes.
A) The morphology of the exosome generated from TAAs loaded DCs was scanned by a TEM (magnification 40,000×).
B) The presence of MHC-II, CD40, CD80, CD86 and CD54 (the classical DC surface markers) was confirmed by western blot on the exosome (a: exosomes generated from DCs loaded with lytic-Ag of BGC823. b: exosomes generated from DCs transfected with total RNA of BGC823)

Dendritic cells loaded with RNA or lytic-Ag from the BGC823 cell line or exosomes derived from these dendritic cells significantly delay tumor growth

To investigate if DCs loaded with RNA or lytic-Ag from the BGC823 cell line or exosomes derived from these DCs could enable therapeutic anti-tumor immunity *in vivo*, we implanted BGC823 cells into 6-8-week-old athymic BALB/c (nu/nu) male mice. The tumors were allowed to establish for 7 days prior to treatment and the mice were sorted with 4 mice per cohort for each group, and the treatment was repeated again after 10 days. After 30 days, as shown in Fig. 5A, we found that the administration of 50 μg of exosome + T cells, DCs transfected with BGC823

cells total RNA + T cell and DCs pulsing with BGC823 cells lytic-Ag + T cell significantly retarded tumor growth in nude mice. As expected, administration of imDCs + T cell or normal saline did not delay tumor growth ($p < 0.05$). And the tumor weight of 50 μg of exosome + T cells, DCs transfected with BGC823 cells total RNA + T cell and DCs pulsing with BGC823 cells lytic-Ag + T cell treated mice was significantly lighter than that of imDCs + T cell or normal saline treated mice (Fig. 5B) ($p < 0.05$).

Discussion

The immunotherapy with DCs for anti-cancer is effective and has a bright future. However, the cancer patient can only produce dysfunctional DCs at an ear-
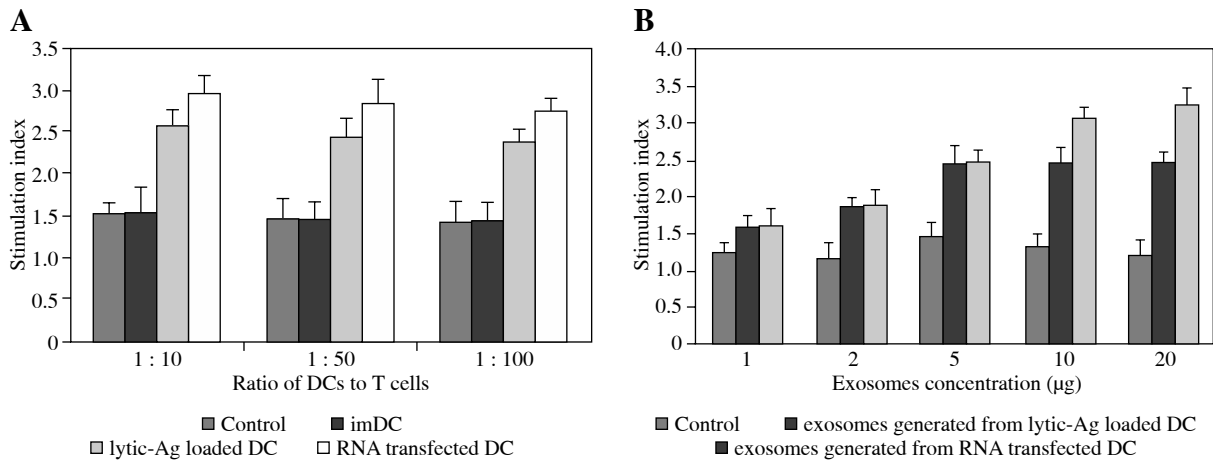
Fig. 3. T-cell proliferation

T cells were (1) co-cultured with lytic Ag-loaded DCs and RNA transfected DCs for 7 days at DCs to T-cell ratio of 1 : 10, 1 : 50, 1 : 100; (2) co-cultured with different concentration exosomes generated from lytic Ag-loaded DCs and RNA transfected DCs (1 μg, 2 μg, 5 μg, 10 μg, 20 μg). Following stimulation, T-cell proliferation was measured by MTT. Results are expressed as a stimulation index. Mean values of 9 experiments, with associated standard error of the means are presented. **A)** Lytic Ag-loaded DCs and RNA transfected DCs efficiently stimulated proliferation of T cells, while T cells co-culture with control or imDCs could not be stimulated to proliferate, $p < 0.05$. **B)** Exosomes generated from lytic Ag-loaded DCs and RNA transfected DCs could efficiently stimulate proliferation of T cells and it was dose-dependent while control and the exosomes generated from imDCs do not have the ability to stimulate T cells proliferation, $p < 0.05$
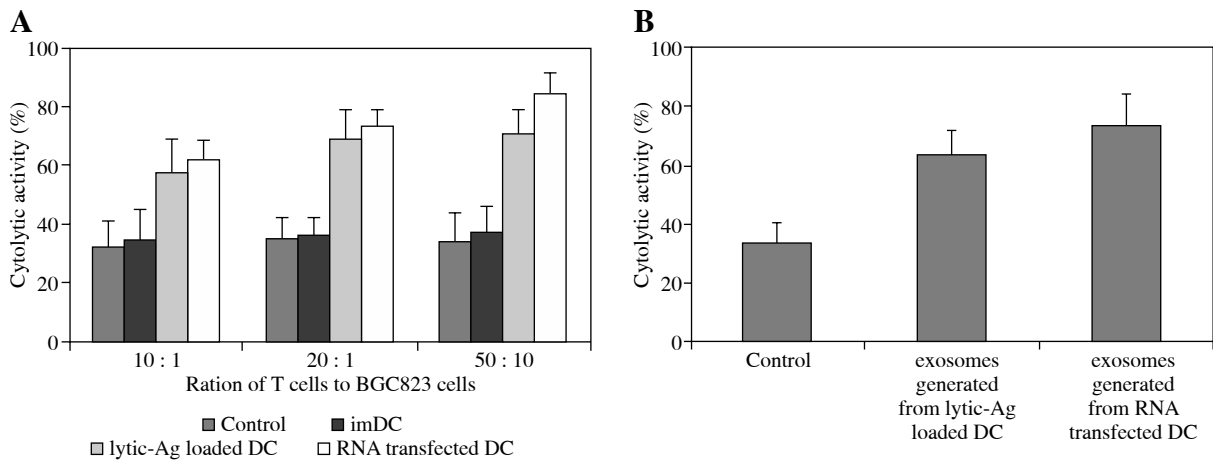

Fig. 4. Cytolytic activity of T cells

T cells were (1) co-cultured with lytic Ag-loaded DCs and RNA transfected DCs for 7 days at DCs to T-cell ratio of 1 : 10, 1 : 50, 1 : 100; (2) co-cultured with 2 μg exosomes generated from lytic Ag-loaded DCs and RNA transfected DCs. Then, cytolytic activity of T cells was performed against BGC823 cells by MTT assay (T cells to BGC823 cells ratio: 10 : 1, 20 : 1, 50 : 1). Experiments were repeated three times, and representative data of similar results are shown. **A)** The cytolytic activity of T cells stimulated by lytic Ag-loaded DCs and RNA transfected DCs could kill BGC823 cells efficiently compared to those T cells unstimulated or T cells stimulated by imDC, $p < 0.01$. **B)** The cytolytic activity of T cells stimulated by 2 μg exosomes could also kill BGC823 cells efficiently compared to those T cells unstimulated

ly stage of cancer. And Gabrilovich *et al.* [10] reported that the deficient antigen-presentation capacity of the DCs found in tumor-bearing animals and in patients with advanced breast cancer induces a cellular-immune deficit. Thus, substitutes for the precursor cells of DCs arouse scientists' interest. Monocytes, isolated from the
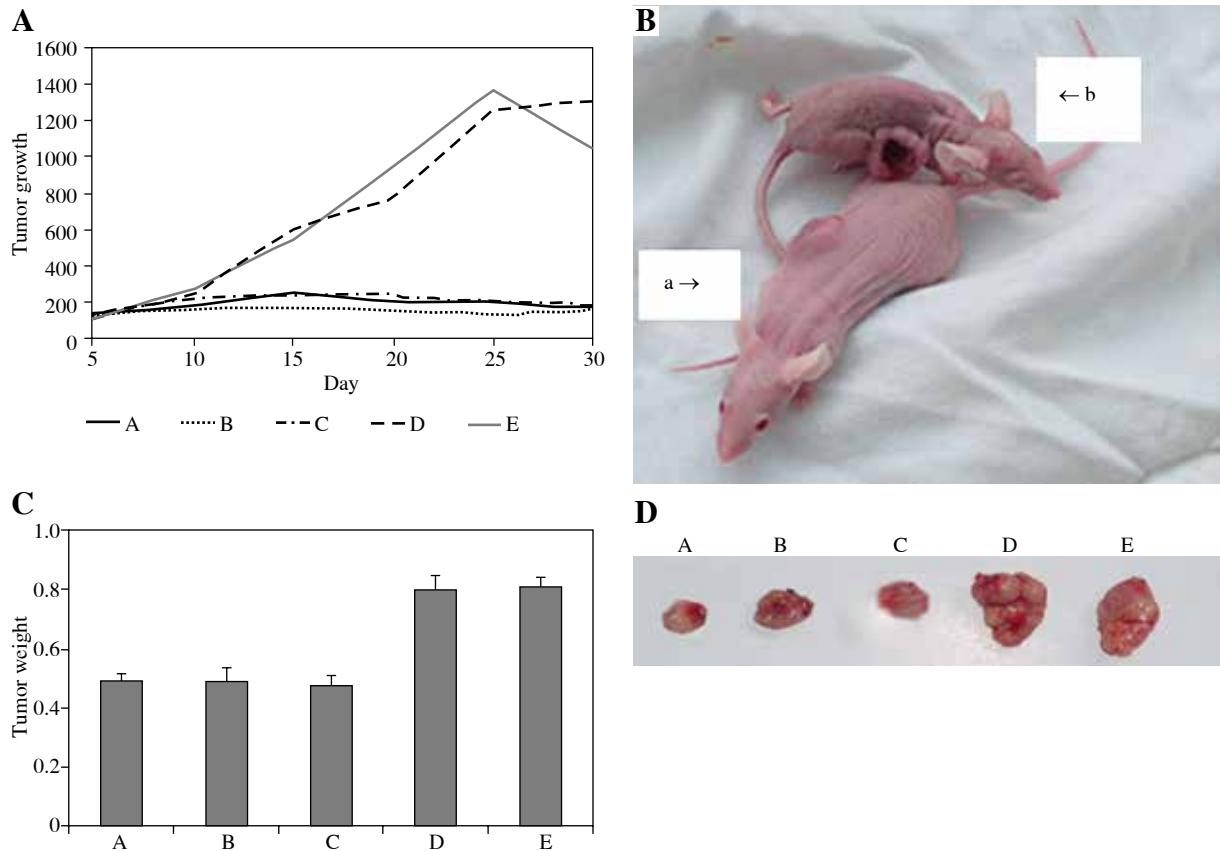
Fig. 5. *In vivo* experiment
Dendritic cell loaded with BGC823 cells tumor and DEex derived from these DCs incubated with T cells together and injected into nude mice xenograft with BGC823 cells. Tumor size was measured every 5 days and the tumor weight was evaluated after the mice were sacrificed. **A)** The tumor growth of mice administered with 50 µg exosome + T cells, DCs transfected with BGC823 cells total RNA + T cell and DCs pulsing with BGC823 cells lytic-Ag + T cell was significantly slower than that of imDCs + T cell or normal sodium ($p < 0.05$). (A: 50 µg exosome + T cells, B: DCs transfected with BGC823 cells total RNA + T cells, C: DCs pulsing with BGC823 cells lytic-Ag + T cells, D: imDCs + T cell, E: normal saline). **B)** Representative photographs of nude mice from the 50 µg exosome + T cells group (a) and the imDCs + T cell group (b). **C)** The T cells, DCs transfected with BGC823 cells total RNA + T cell and DCs pulsing with BGC823 cells lytic-Ag + T cell was significantly slower than that of imDCs + T cell or normal sodium ($p < 0.05$). **D)** Representative photographs of tumor blocks from the five groups

patients' peripheral blood, were regarded as one of the substitutes, but cannot produce enough or expected DCs. In the present study, we isolated monocytes from cord blood using differential centrifugation, and cultured in culture flask for 2 h, the adherent cells were the precursor cells of DCs and then they were cultured with RPMI 1640 supplemented with rhSCF, rhGM-CSF and rhIL-4. At day 10, the number of DCs can be amplified 10 times as before and typical morphous of DCs and surface markers occurred.

Only DCs with antigen can present antigen to T cells efficiently, and can induce specific antitumor activity. Incubation of DCs with tumor specific molecules such as proteins, peptides, or lysates, or transfecting them with

nucleic acids encoding tumor specific antigens is the basic concept of designing DC-based cancer immunotherapy [11-14]. In this study, DCs from UCB were loaded with BGC823 cell tumor antigens by transfecting total of RNA BGC823 cell or pulsing with tumor antigens generated from the BGC823 cell line, and had a high expression level for molecules related to DCs maturation, especially for DCs transfected with BGC823 cell total RNA. We found the evidence that DCs with the tumor antigens could prime T cells to generate the tumor-specific CTLs, which can kill BGC823 cell.0

Dendritic cells also produce exosomes, potentially carrying DCs transmembrane molecules and cytokines, and hence they have some functions of DCs. They are superior

than DCs in that they are tolerant to high temperature, easy to store, stable, molecularly defined and easy to perform quantitative analysis [15]. With immuno-regulatory roles and potent antitumor effects, easy to access, they have been a potential source on antitumor biotherapy. It is not unusual to see the reports that UBMC could be induced to DCs *in vitro*. However, there have been no other reports concerning the exosomes derived from UBDC.

In the present study, differential centrifugation method is used to isolate exosomes from the supernatant of tumor antigen-loaded DC from cord blood. Systemically characterized by TEM or flow cytometry, the phenotype of DCex was coincidence with what was reported previously. We demonstrated that exosomes expressed MHC-II, CD40, CD80, CD86 and CD54, indicating that exosomes carry all the immunologically important molecules of DC to induce immune responses. A further study showed that exosomes derived from BGC823 protein-pulsed exosomes could stimulate T cell proliferation and enhance the cytotoxic activity of CTL *in vitro*. Thus, exosomes derived from tumor antigen loaded DCs may represent a novel and feasible exosome-based vaccine approach against tumors.

As reported previously, as a tumor vaccine, exosomes could prevent tumor development, diminish or even regress the established tumor in experimental animals [16]. Similar to mature DCs, tumor antigens pulsing exosomes vaccine can stimulate special CTL and inhibit tumor growth. It can leave the antigen peptide vaccine far behind [17]. In this study, human gastric adenocarcinoma cell line BGC823 cells were injected into nude mouse to establish the animal model. After tumor was formed, mature DCs and exosomes were injected around the tumor mass. Because of deficiency of T cells, the nude mouse should be injected with T cells and IL-2 at the same time. We found that DCs and exosomes all could activate T cells to perform anti-tumor activity. Obviously, cell-free products, exosomes, offer a number of advantages compared with DCs transplantation. Cell-free products can be standardized and tested in terms of dose and biological activity. Exosomes could be stored without potentially toxic cryopreservatives at –20°C for 6 months with no loss in their biochemical activities. Exosomes, therefore, exhibit several attractive features as a therapeutic agent.

In short, we have shown that UCB was a rich source for DCs and DCs with tumor antigens were highly efficient methods for immunotherapy *in vitro* and *in vivo*. Our data also show that DCex expressing a higher level of MHC-II and costimulatory CD40, CD80, CD86 and CD54 molecules could efficiently stimulate T cells proliferation and cytotoxicity *in vitro* and *in vivo*. And because of its advantages beyond DCs, exosomes may become a more effective DC-based vaccine for the induction of antitumor immunity.

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