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Tumor cell-derived exosome-targeted dendritic cells stimulate stronger $CD8^+$ CTL responses and antitumor immunities

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ABSTRACT

Tumor cell-derived exosomes (TEX) have been widely used to induce antitumor immune responses in animal models and clinical trials. However, the efficiency of the antitumor immunity that is induced by TEX is still relatively weak. In this study, we compared the antitumor immunities between EG7 tumor cell-derived exosomes (EXO_{EG7}) and EXO_{EG7}-targeted dendritic cells (DC_{EXO}). We found that EXO_{EG7} harbored OVA and peptide major histocompatibility complex I (pMHC-I), which were expressed on its parental EG7 tumor cells, and they could transfer OVA and pMHC-I to dendritic cells (DCs) *in vitro*. DC_{EXO} could more efficiently induce antitumor immunity than EXO_{EG7}. In addition, we showed that the immune stimulatory effects of EXO_{EG7} were dependent on the host DCs and, whereas those of DC_{EXO} were not, indicating the important role of the host DCs in TEX vaccines. Taken together, TEX-targeted DCs may be more effective for EXO-based vaccines for the induction of antitumor immunity.

1. Introduction

Tumor cells express a series of antigens that are recognized by cytotoxic T lymphocytes (CTL) [3,23]. However, most of the special tumor antigens have not been identified, and the nature of the tumor antigens that mediate efficient immune responses that lead to tumor rejection remains unclear [13].

One general characteristic of tumor cells is the releasing or shedding of membrane vesicles, which are called exosomes (EXO). EXOs are small membrane-bound vesicles that are released by a variety of cell types [10,28]. Many types of cells, such as dendritic cells (DCs) and B lymphocytes, as well as various tumor cells, that excrete EXOs are of increased interest in tumor immunotherapy [29,33]. Functional analyses have shown that EXOs that are derived from antigen-presenting cells contain a number of antigen-presenting molecules, such as major histocompatibility complex class I and II (MHC-I and MHC-II), costimulatory molecules (CD80 and CD86), and heat shock proteins 70 and 90 (HSP70/HSP90) [11,27]. Some reports have shown that DC-derived EXOs (EXO_{DC}) have antigen-presenting capabilities, which make them a potentially attractive vehicle for immunotherapy [14,18]. Recently, reports from Altieri, Andre and Wolfers have demonstrated that tumor cell-derived exosomes (TEXs) and those isolated from malignant effusions can induce antigen-specific CTL responses and antitumor immunities [1,2,29]. Thus, TEXs have attracted much attention as a potential source of tumor antigens [5,20].

Zitvogel and our previous studies have shown that peptide-pulsed DCs and EXO_{DC} elicit potent antitumor immune responses [15,33].However, the efficiency of the antitumor responses induced by EXO_{DC} is higher than that of EG7 tumor cell-derived EXO (EXO_{EG7}), and this is perhaps due to the lower levels of expression of MHC-II and costimulatory molecules on EXO_{EG7} compared to the expression on EXO_{DC}. In another study, we have demonstrated that EXO_{DC} target other DCs *in vitro*, and EXO_{DC}-targeted DCs can more strongly stimulate antigen-specific $CD8^+$ T-cell proliferation *in vitro* and *in vivo* and more efficiently induce antigen-specific CTL responses and antitumor immunities than EXO_{DC} [14]. Therefore, it is hypothesized that TEXs may transfer tumor antigens to DCs and that TEX-targeted DCs may more efficiently induce antitumor immunity.

In this study, we first investigated the phenotype characteristics of EXO_{EG7} with flow cytometry and compared the efficiency of EXO_{EG7} and EXO_{EG7}-targeted DCs in the stimulation of antigen-specific CTL responses and antitumor immunity.

2. Materials and methods

2.1. Reagents, cell lines, and animals

OVA was obtained from Sigma–Aldrich China, Inc. (Shanghai, China). Biotin or fluorescein isothiocyanate (FITC)-labeled antibodies (Abs) specific for H-$2K^b$, Ia^b, CD40, CD54, or CD80 were all

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obtained from Becton Dickinson Medical Devices Co., Ltd. (Shanghai, China). Anti-Mouse OVA257-264 (SIINFEKL) peptide that was bound to H-2K^b PE-Cy7 was purchased from eBioscience (Shanghai, China). The anti-H-2K^b/OVA-I (pMHC-I) complex Ab was produced by the Chinese Peptide Company (Hangzhou, China). Mouse recombinant IL-4 and GM-CSF were purchased from PeproTech (Shanghai, China). The carboxyfluorescein succinimidyl ester (CFSE) was obtained from Invitrogen China Limited (Shanghai, China). A CytoTox96 Non-Radioactive Cytotoxicity Assay Kit was purchased from Shanghai Promega Biological Products, Ltd. (Shanghai, China). The OVA-transfected tumor cell line EG7 (H-2b), BL6-10$_{OVA}$ melanoma cell lines, and wild-type mouse thymoma tumor cell line EL-4 (H-2b) were generated in the Shanghai Laboratory Animal Center (Shanghai, China). Female C57BL/6 and the diphtheria toxin receptor (DTR)-transgenic mice were obtained from the Shanghai Laboratory Animal Center and used at 6–14 weeks. They were allowed to adapt to their environment for 1 week before the initiation of the experiments, and all animals were maintained under standard environmental conditions with free access to food and water. Mice were treated according to the animal care committee guidelines of the School of Medicine of Shanghai Jiaotong University.

2.2. Generation of bone marrow-derived DCs

The generation of bone marrow-derived DCs has been described previously [16]. Briefly, bone marrow cells prepared from the femora and tibiae of naive C57BL/6 mice were depleted of red blood cells with 0.84% ammonium chloride and plated in DC culture medium containing 10% FCS, GM-CSF (10 ng/mL), and IL-4 (10 ng/mL). On day 3, the nonadherent granulocytes and T and B lymphocytes were gently removed and fresh media was added, and, 2 days later, the DCs aggregates were dislodged and replated. On day 7, DCs were harvested. To generate OVA-pulsed DCs (DC$_{OVA}$), DCs were pulsed with OVA protein (0.3 mg/mL) in AIM-V medium (GIBCO) for overnight culture.

2.3. Generation and purification of exosomes

EXOs were isolated as described previously [6,25]. Briefly, the supernatants of EG7 cells were subjected to 4 successive centrifugations at 300*g* for 5 min and 1200*g* for 20 min and 10,000*g* for 30 min to remove cells and cellular debris, and 100,000*g* for 1 h to pellet EXOs. The EXO pellets were washed twice in a large volume of PBS and recovered by centrifugation at 100,000*g* for 1 h. The amount of exosomal proteins recovered was measured using Bradford assay. EG7 cell-derived exosomes were defined as EXO$_{EG7}$.

2.4. Phenotypic characterization of EG7 cells and EXO$_{EG7}$

For the phenotypic analysis of EG7 cells and EXO$_{EG7}$, both EG7 cells and EXO$_{EG7}$ were stained with a panel of biotin-labeled and FITC-labeled Abs and analyzed by flow cytometry with FACScan (Coulter EPICS XL, Beckman Coulter, Inc., Brea, CA) as previously described [8,14].

2.5. Exosomes taken up by DCs

To test whether EXO$_{EG7}$ could be taken up by DCs *in vitro*, DCs were co-cultured with CFSE-labeled EXO$_{EG7}$ (EXO$_{CFSE}$) for 4 h, and CFSE-positive cells were then detected by flow cytometry [14]. To further confirm that EXO transferred its molecules to DCs, DCs were analyzed for the expression of OVA and the pMHC-I molecule after incubating with EXO$_{EG7}$. DCs pulsed (targeted) with EXO$_{EG7}$ were defined as DC$_{EXO}$.

2.6. Tetramer staining

C57BL/6 mice were intravenously immunized with EXO$_{EG7}$ (10 μg/mouse) or DC$_{EXO}$ (1×10^6/mouse). Six days later, tail blood was harvested and incubated with 10 μL of the PE-H-2K^b/OVA$_{257-264}$ tetramer (eBioscience) and FITC-CD8 for 30 min at room temperature, and the cells were then analyzed by flow cytometry. To investigate the involvement of the host DCs in the TEX vaccines, we also used DTR-transgenic mice, in which CD11c$^+$ DCs were sensitive to diphtheria toxin (DT) [19]. The DTR-transgenic and wild-type C57BL/6 mice were intraperitoneally injected with 2 doses of DT (1.5 ng/g weight) every 3 days. After 2 days, almost no CD11c$^+$ DCs were detectable in the spleen (data not shown) [19]. The treated mice were then intravenously injected with EXO$_{EG7}$ or DC$_{EXO}$, and CD8$^+$ T cells expressing the H-2K^b/OVA$_{257-264}$ tetramer were detected.

2.7. Cytotoxicity assay

In vitro cytotoxicity was analyzed with the lactate dehydrogenase (LDH) releasing method with the CytoTox96 Cytotoxicity Assay Kit according to the manufacturer's instructions. Spleen T lymphocytes from the above immunized mice were harvested with nylon wool columns after 7 days of immunization. Spleen T lymphocytes (5 × 106) were co-cultured with γ-irradiated (6000 rad) EG7 cells (1 × 105) in Dulbecco's Modified Eagle's Medium plus 10% FCS containing IL-2 (20 U/mL) in a 24-well plate (Costar, Shanghai, China). Five days later, T cells were harvested and used as effectors. This enzymatic assay colorimetrically measured the amount of LDH that was released from lysed target cells, including EG7 or control EL-4 cells that were mixed with different ratios of effectors for 4 h at 37 °C. The spontaneous/maximal release ratio was <20% in all experiments. Specific lyses (%) was calculated with the following formula: (experimental LDH release − effectors spontaneous LDH release − target spontaneous LDH release)/(target maximum LDH release) × 100.

2.8. Animal studies

To examine whether EXO$_{EG7}$ or DC$_{EXO}$ induced antitumor protective immunity, wild-type C57BL/6 and DC-knockout mice ($n = 8$) were intravenously injected with EXO$_{EG7}$ (10 μg/mouse) and DC$_{EXO}$ (1×10^6/mouse), respectively. After 6 days, the immunized mice were intravenously challenged with BL6-10$_{OVA}$ (0.5×10^6/mouse). To investigate the involvement of the host DC in EXO vaccines, we also used diphtheria toxin receptor (DTR) transgenic mice, in which, CD11c$^+$ DC sensitive to diphtheria toxin (DT) [19]. The DTR transgenic and wild-type C57BL/6 mice were injected single dose of DT (1.5 ng/g weight), after 2 days, almost no CD11c$^+$ DC were detectable in spleen of DTR transgenic mice [19]. The treated mice ($n = 8$) were then injected i.v. with EXO$_{EG7}$ (10 μg/mouse) and DC$_{EXO}$ (1×10^6/mouse), respectively, and then i.v. challenged with BL6-10$_{OVA}$ (0.5×10^6/mouse) 6 days later. The mice were sacrificed 4 weeks after the tumor cell injection, and the lung metastatic tumor colonies were counted in a blind fashion. Metastases on freshly isolated lungs appeared as discrete black pigmented foci that were easily distinguishable from normal lung tissues, and they were confirmed with histological examination. Metastatic foci that were too numerous to count were assigned an arbitrary value of >100 [30].

3. Results

3.1. Phenotypic characterization of EG7 tumor cells and EXO$_{EG7}$

EG7 tumor cells expressed MHC-class I, CD54, and OVA, but MHC-class II, CD40, and CD80 were almost undetectable. In

addition, EG7 tumor cells also expressed pMHC-I (Fig. 1). Similar to EG7 tumor cells, EXO_{EG7} also expressed MHC-class I, OVA, CD54, and pMHC-I but at a lower level than EG7 tumor cells, and they did not express MHC-class II, CD40, and CD80 molecules, indicating that EXO_{EG7} harbored the membrane molecules and tumor cell-associated antigens.

3.2. EXO_{EG7} transferred exosomal molecules to DCs

To examine if TEX could target DCs and transfer its molecules onto DCs, DCs were co-cultured with EXO_{CFSE} for 4 h and examined by fluorescence microscopy. As shown in Fig. 2a, the CFSE-positive cells were detectable after being incubated with EXO_{CFSE}. To further confirm exosomal molecules were transferred to DCs, DCs were co-cultured with EXO_{EG7} and then analyzed for the expression of OVA and pMHC-I molecules. As shown in Fig. 2b, OVA and pMHC-I were detectable on DCs that did not express OVA and pMHC-I, indicating that DCs can uptake EXO_{EG7} and acquire exosomal OVA and pMHC-I, which is critical in the stimulation of OVA-specific CTL responses.

3.3. DC_{EXO} stimulated stronger $CD8^+$ T-cell proliferation

Although EXO_{EG7} were found to transfer OVA and pMHC-I molecules to DCs, it was unclear whether EXO_{EG7} stimulated $CD8^+$ T-cell proliferation *in vivo*. As shown in Fig. 3a, EXO_{EG7} immunization induced 0.48% tetramer-positive $CD8^+$ T cells, indicating that EXO_{EG7} activated naïve OVA-specific $CD8^+$ T cells *in vivo* at a low level. Interestingly, DC_{EXO} stimulated 1.14% tetramer-positive $CD8^+$ T-cell responses, which was greater than that of EXO_{EG7}, but no significant difference was observed between mice immunized with DC_{EXO} and DC_{OVA} (1.58%). No tetramer-positive $CD8^+$ T cells were detected in mice immunized with unpulsed DCs and DCs pulsed with EL4-derived exosomes (data not shown), indicating that EXO_{EG7} and DC_{EXO} induced OVA-specific CTL responses. In addition, our data showed that the diphtheria toxin (DT) treatment itself did not affect the tetramer-positive $CD8^+$ T cell responses since a similar amount of the tetramer-positive $CD8^+$ T cell responses (data not shown) was detected in DT-treated C57BL/6 mice as seen in untreated C57BL/6 mice. However, no tetramer-positive $CD8^+$ T cells were detected in DT-treated DTR-transgenic mice immunized with EXO_{EG7}, but DC_{EXO} immunization did induce 0.81% tetramer-positive $CD8^+$ T cells in DC-depleted mice (Fig. 3b), indicating that the induction of $CD8^+$ T cell activation by EXO_{EG7} was dependent upon host DCs, and not DC_{EXO}.

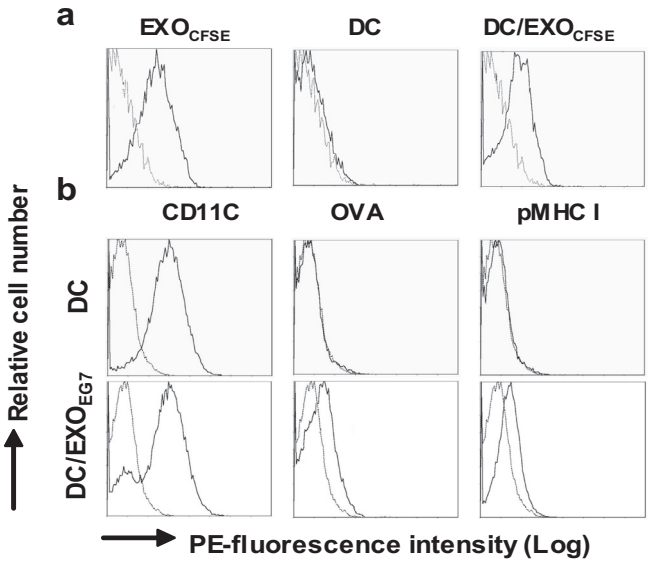

Fig. 2. Exosome (EXO) uptaken by dendritic cells (DCs). (A) To test if EXO transferred molecules onto DCs, DCs were co-cultured with carboxyfluorescein succinimidyl ester (CFSE)-labeled EXO_{EG7} (EXO_{CFSE}) for 4 h and then examined with fluorescence microscopy. (B) To test if EXO molecules transferred to DCs, DCs that were not pulsed with OVA and expressed CD11C but not OVA and pMHC-I (upper panel) were co-cultured with EXO_{EG7}, and then analyzed for OVA and pMHC-I expression by flow cytometry.

3.4. DC_{EXO} stimulated stronger $CD8^+$ T-cell differentiation into CTL effectors

Next, we tested the ability of EXO_{EG7} and DC_{EXO} to induce the differentiation of $CD8^+$ T cells into CTL effectors with the LDH releasing method. As shown in Fig. 4, spleen T cells from mice immunized with EXO_{EG7} *in vitro* displayed killing activities against EG7 cells (24% killing; E:T ratio, 25:1), but this was much weaker than those activated by DC_{OVA} and DC_{EXO} (52.15% and 49.23% killing; E:T ratio, 25:1), indicating that DC_{EXO} stimulated strong $CD8^+$ T cell differentiation into CTL effectors. No killing activities against its parental EL4 tumor cells were detectable, indicating that the killing activity of these CTLs was OVA specific.

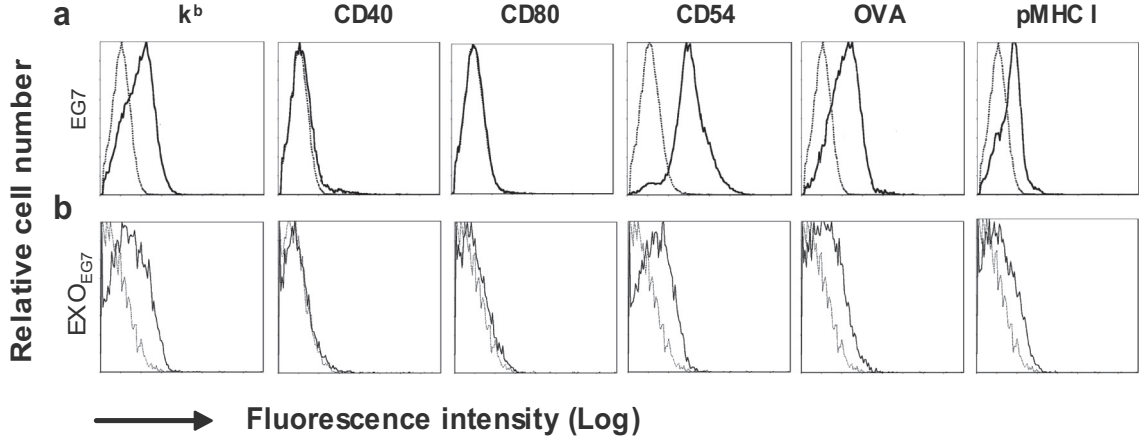

Fig. 1. Phenotypic analysis of EG7 cells and EG7 tumor cell-derived exosomes (EXO_{EG7}). EG7 tumor cells and EXO_{EG7} (solid lines) were stained with a panel of antibodies (Abs) and then analyzed with flow cytometry. The cells and EXO_{EG7} (thin dotted lines) were also stained with isotype-matched irrelevant Abs and employed as control populations. One representative experiment of 2 is displayed.

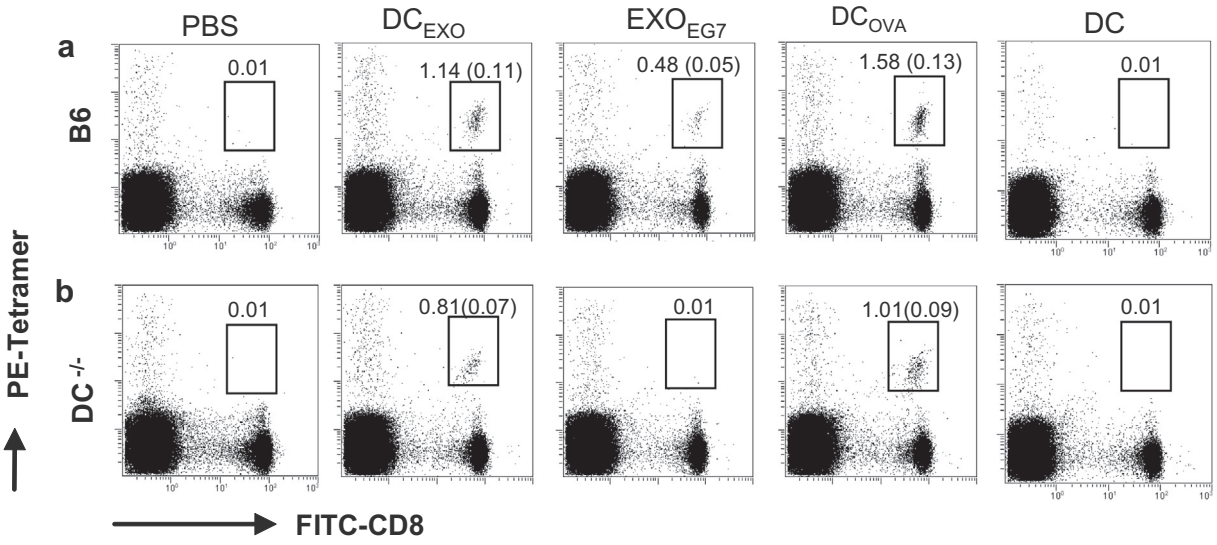

Fig. 3. Stimulation of T-cell proliferation *in vivo*. C57BL/6 and diphtheria toxin receptor (DTR)-transgenic mice were intravenously immunized with EXO_{EG7}-targeted dendritic cells (DC_{EXO}) and EXO_{EG7}, respectively. Six days after immunization, tail blood samples were taken from (A) the immunized C57BL/6 mice or (B) the immunized DTR-transgenic mice treated with diphtheria toxin and stained with the PE-H-2K^b/OVA tetramer and FITC-anti-CD8 Ab. The expression of PE-H-2K^b/OVA tetramer-specific TCR and CD8 molecules was examined with flow cytometry. The results presented are representative of 4 separate mice per group. The values in parentheses represent the standard deviation. One representative experiment of three is shown.

3.5. DC_{EXO} induced stronger antitumor immunity

Next, we investigated the ability of EXO_{EG7} and DC_{EXO} to induce antitumor immunity *in vivo*. As shown in Exp. I of Table 1, all the mice that were injected with PBS had large numbers (>100) of lung metastatic tumor colonies. Seven out of 8 (88%) mice with DC_{EXO} immunization were tumor-free, whereas only 4 out of 8 (50%) mice were tumor-free in the EXO_{EG7} group, indicating that DC_{EXO} more efficiently induced antitumor protective immunity than did EXO_{EG7}. The specificity of the protection was confirmed with the observation that both DC_{EXO} and EXO_{EG7} did not protect against BL6-10 tumors that did not express OVA, with all mice having large numbers (>100) of lung metastatic tumor colonies after the tumor cell challenge. DT treatment did not affect the OVA-specific antitumor immunity that was derived from EXO_{EG7} vaccination because a similar extent of antitumor immunity (data not shown) was found in DT-treated mice as that seen in untreated mice. Interestingly, our data also showed that all EXO_{EG7}-immunized DC depleted mice by DT treatment had large numbers (>100) of lung metastatic tumor colonies. However, immunization with DC_{EXO} and DC_{OVA} protected 6/8 (75%) of the mice against BL6-10_{OVA} tumor cells (Exp. II of Table 1), indicating that the antitumor immunity that was induced by EXO_{EG7} depended upon the host DC.

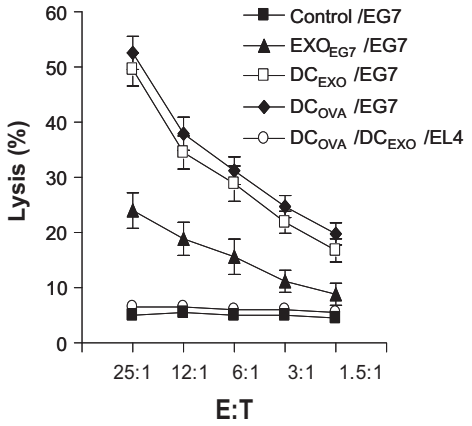

Fig. 4. Development of antigen-specific cytotoxic T lymphocytes (CTL) activities. In an *in vitro* cytotoxicity assay, spleen T lymphocytes from the immunized mice were harvested after 7 days of immunization and co-cultured with irradiated (6000 rad) EG7 cells (1×10^5) in a 24-well plate. After 5 days, the harvested T cells were used as effectors (E) in a non-radioactive cytotoxicity assay, whereas EG7 or control EL-4 cells were used as target (T) cells. This enzymatic assay colorimetrically measured the amount of lactate dehydrogenase (LDH) that was released from lysed target cells. Specific lysis (%) was calculated with the following formula: (experimental LDH release − effector spontaneous LDH release − target spontaneous LDH release)/(target maximum LDH release) × 100. Each point represents the mean of triplicate cultures. One representative experiment of three is shown.

4. Discussion

EXO are membrane-bound vesicles that are released into the extracellular space when a multivesicular body fuses with the plasma membrane [24]. EXO contain cytosolic and membrane proteins that are derived from the parental cells [26,31]. It has been demonstrated that EXO_{DC} harbor many important immunological molecules and that they can elicit antitumor immunity [2,6,14].

TEXs can induce antigen-specific CTL responses and antitumor protective immunity [1,9,32]. However, the efficiency of the antitumor immunity of TEX is relatively weak [15,17]. Recently, some studies reported that the physiopathological role of TEXs might be more in favor of immune suppression and tumor promotion [7,12]. Therefore, disadvantages and dangers may remain in TEX-based vaccines, and efforts should be taken to overcome the disadvantageous position of TEX-based vaccines. In a previous study, we demonstrated that EXO_{DC} can transfer exosomal molecules to DCs, and EXO_{DC}-targeted DCs can more strongly stimulate OVA-specific CD8$^+$ T-cell proliferation *in vitro* and *in vivo* and more efficiently induce OVA-specific CTL responses, antitumor immunity, and CD8$^+$ T-cell memory *in vivo* compared to EXO_{DC} [14].

In this study, we demonstrated that EXO_{EG7} harbor OVA and pMHC-I that are expressed on its parental tumor cells, which is consistent with the findings of previous studies. Knight et al. have shown that DCs acquire antigens from cell-free DCs supernatants [21]. In this study, we showed that EXO_{EG7} were taken up by DCs *in vitro*, and the OVA antigen and pMHC-I were transferred to

Table 1
EXO_{EG7} and DC_{EXO} protect against tumor metastases.

Vaccines	Tumor cell challenge	Tumor growth incidence (%)	Median number of lung tumor colonies
Exp. I. B6 mice			
EXO_{EG7}	BL6-10_{OVA}	4/8 (50)	29 ± 6*
DC_{EXO}	BL6-10_{OVA}	1/8 (13)	5 ± 2
DC_{OVA}	BL6-10_{OVA}	0/8 (0)	0
PBS	BL6-10_{OVA}	8/8 (100)	>100
DC	BL6-10_{OVA}	8/8 (100)	>100
$EG7_{EXO}$	BL6-10	8/8 (100)	>100
DC_{EXO}	BL6-10	8/8 (100)	>100
Exp. II. $DC^{-/-}$ mice			
$EG7_{EXO}$	BL6-10_{OVA}	8/8 (100)	>100
DC_{EXO}	BL6-10_{OVA}	2/8 (25)	11 ± 5
DC_{OVA}	BL6-10_{OVA}	2/8 (25)	9 ± 3
PBS	BL6-10_{OVA}	8/8 (100)	>100

In experiment I, C57BL/6 were i.v. immunized with $EG7_{EXO}$ and DC_{EXO}. Six days later, each mouse was challenged i.v. with BL6-10_{OVA} or BL6-10 tumor cells. In experiment II. C57BL/6 mice with DC deletion ($n = 8$) were i.v. immunized with $EG7_{EXO}$ and DC_{EXO}. Six days later, each mouse was challenged i.v. with BL6-10_{OVA} tumor cells.
* $p < 0.05$ (nonparametric Mann–Whitney U test) vs cohorts of mice immunized with the same number of DC_{OVA}. The mice were sacrificed 4 weeks after tumor cell challenge and the numbers of lung metastatic tumor colonies were counted. One representative experiment of 3 is shown.

DCs. In addition, we also showed that DC_{EXO} immunization induced 1.14% tetramer-positive $CD8^+$ T cells, and T cells from DC_{EXO}-immunized mice displayed stronger killing activity (49.23% killing; E:T ratio, 25:1) against EG7 cells and induced more efficient (87%) immune protection compared to EXO_{EG7}. These data first demonstrated that TEX-targeted DCs could stimulate stronger $CD8^+$ T-cell proliferation and differentiation into CTL effectors *in vivo* and induce stronger antitumor protective immunity against tumor cell challenges compared to TEX. The decreased immunogenicity of TEX compared to TEX-targeted DCs may possibly be due to the absence of the expression of the costimulatory molecules CD40 and CD80 on TEX, both molecules have been repeatedly shown to be key elements in the initiation of primary immune responses [22].

The *in vivo* mechanisms by which EXO induces immunity is still unknown. It has been previously reported that EXO may need the host DC for the induction of immune responses based only upon the results of *in vitro* experiments [4]. However, there is no direct evidence for the role of the host DC in EXO-based vaccines. In this study, we demonstrated that EXO_{EG7} completely depended on the host DC in the induction of antitumor immunity, and, for the first time, we provided clear evidence that, similar to antigen-pulsed DC, EXO_{EG7}-targeted DCs do not need host DC for the induction of their antitumor immunity.

Because TEX carry tumor antigens, TEX-targeted DC vaccines may be feasible for combating tumors with EXOs that are purified from the cancer patient's ascites [2], which are then taken up by the *in vitro*-activated DCs that are derived from the patient's peripheral blood monocytes. Thus, TEX-targeted DC vaccines could represent a novel and feasible TEX- and DC-based immunotherapy for tumors.

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References

[1] S.L. Altieri, A.N. Khan, T.B. Tomasi, Exosomes from plasmacytoma cells as a tumor vaccine, J. Immunother. 27 (2004) 282–288.
[2] F. Andre, N.E. Schartz, M. Movassagh, C. Flament, P. Pautier, P. Morice, C. Pomel, C. Lhomme, B. Escudier, T. Le Chevalier, et al., Malignant effusions and immunogenic tumour-derived exosomes, Lancet 360 (2002) 295–305.
[3] T. Boon, P. van der Bruggen, Human tumor antigens recognized by T lymphocytes, J. Exp. Med. 183 (1996) 725–729.
[4] N. Chaput, N.E. Schartz, F. Andre, J. Taieb, S. Novault, P. Bonnaventure, N. Aubert, J. Bernard, F. Lemonnier, M. Merad, et al., Exosomes as potent cell-free peptide-based vaccine. II. Exosomes in CpG adjuvants efficiently prime naive Tc1 lymphocytes leading to tumor rejection, J. Immunol. 172 (2004) 2137–2146.
[5] N. Chaput, N.E. Schartz, F. Andre, L. Zitvogel, Exosomes for immunotherapy of cancer, Adv. Exp. Med. Biol. 532 (2003) 215–221.
[6] J.A. Cho, D.J. Yeo, H.Y. Son, H.W. Kim, D.S. Jung, J.K. Ko, J.S. Koh, Y.N. Kim, C.W. Kim, Exosomes: a new delivery system for tumor antigens in cancer immunotherapy, Int. J. Cancer 114 (2005) 613–622.
[7] A. Clayton, S. Al-Taei, J. Webber, M.D. Mason, Z. Tabi, Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production, J. Immunol. 187 (2011) 676–683.
[8] A. Clayton, J. Court, H. Navabi, M. Adams, M.D. Mason, J.A. Hobot, G.R. Newman, B. Jasani, Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry, J. Immunol. Methods 247 (2001) 163–174.
[9] S. Dai, D. Wei, Z. Wu, X. Zhou, X. Wei, H. Huang, G. Li, Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer, Mol. Ther.:J. Am. Soc. Gene Ther. 16 (2008) 782–790.
[10] K. Denzer, M.J. Kleijmeer, H.F. Heijnen, W. Stoorvogel, H.J. Geuze, Exosome: from internal vesicle of the multivesicular body to intercellular signaling device, J. Cell Sci. 113 (Pt.19) (2000) 3365–3374.
[11] J.M. Escola, M.J. Kleijmeer, W. Stoorvogel, J.M. Griffith, O. Yoshie, H.J. Geuze, Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes, J. Biol. Chem. 273 (1998) 20121–20127.
[12] P. Filipazzi, M. Burdek, A. Villa, L. Rivoltini, V. Huber, Recent advances on the role of tumor exosomes in immunosuppression and disease progression, Sem. Cancer Biol. 22 (2012) 342–349.
[13] E. Gilboa, The makings of a tumor rejection antigen, Immunity 11 (1999) 263–270.
[14] S. Hao, O. Bai, F. Li, J. Yuan, S. Laferte, J. Xiang, Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity, Immunology 120 (2007) 90–102.
[15] S. Hao, O. Bai, J. Yuan, M. Qureshi, J. Xiang, Dendritic cell-derived exosomes stimulate stronger CD8+ CTL responses and antitumor immunity than tumor cell-derived exosomes, Cell. Mol. Immunol. 3 (2006) 205–211.
[16] S. Hao, Y. Liu, J. Yuan, X. Zhang, T. He, X. Wu, Y. Wei, D. Sun, J. Xiang, Novel exosome-targeted CD4+ T cell vaccine counteracting CD4+25+ regulatory T cell-mediated immune suppression and stimulating efficient central memory CD8+ CTL responses, J. Immunol. 179 (2007) 2731–2740.
[17] S. Hao, Z. Ye, J. Yang, O. Bai, J. Xiang, Intradermal vaccination of dendritic cell-derived exosomes is superior to a subcutaneous one in the induction of antitumor immunity, Cancer Biother. Radiopharm. 21 (2006) 146–154.
[18] I. Hwang, X. Shen, J. Sprent, Direct stimulation of naive T cells by membrane vesicles from antigen-presenting cells: distinct roles for CD54 and B7 molecules, Proc. Natl. Acad. Sci. USA 100 (2003) 6670–6675.
[19] S. Jung, D. Unutmaz, P. Wong, G. Sano, K. De los Santos, Sparwasser T., Wu S., Vuthoori S, Ko K., Zavala F, *in vivo* depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens, Immunity 17 (2002) 211–220.
[20] J.V. Kim, J.B. Latouche, I. Riviere, M. Sadelain, The ABCs of artificial antigen presentation, Nat. Biotechnol. 22 (2004) 403–410.
[21] S.C. Knight, S. Iqball, M.S. Roberts, S. Macatonia, P.A. Bedford, Transfer of antigen between dendritic cells in the stimulation of primary T cell proliferation, Eur. J. Immunol. 28 (1998) 1636–1644.
[22] N. Mazouz, A. Ooms, V. Moulin, S. Van Meirvenne, C. Uyttenhove, G. Degiovanni, CD40 triggering increases the efficiency of dendritic cells for antitumoral immunization, Cancer Immunol. 2 (2002) 2.
[23] S.A. Rosenberg, A new era for cancer immunotherapy based on the genes that encode cancer antigens, Immunity 10 (1999) 281–287.

[24] M. Simons, G. Raposo, Exosomes–vesicular carriers for intercellular communication, Curr. Opin. Cell Biol. 21 (2009) 575–581.
[25] C. Thery, L. Duban, E. Segura, P. Veron, O. Lantz, S. Amigorena, Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes, Nat. Immunol. 3 (2002) 1156–1162.
[26] C. Thery, M. Ostrowski, E. Segura, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (2009) 581–593.
[27] C. Thery, A. Regnault, J. Garin, J. Wolfers, L. Zitvogel, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73, J. Cell Biol. 147 (1999) 599–610.
[28] C. Thery, L. Zitvogel, S. Amigorena, Exosomes: composition, biogenesis and function, Nat. Rev. Immunol. 2 (2002) 569–579.
[29] J. Wolfers, A. Lozier, G. Raposo, A. Regnault, C. Thery, C. Masurier, C. Flament, S. Pouzieux, F. Faure, T. Tursz, et al., Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming, Nat. Med. 7 (2001) 297–303.
[30] J. Xiang, H. Huang, Y. Liu, A new dynamic model of CD8+ T effector cell responses via CD4+ T helper-antigen-presenting cells, J. Immunol. 174 (2005) 7497–7505.
[31] C. Yang, P.D. Robbins, The roles of tumor-derived exosomes in cancer pathogenesis, Clin. Dev. Immunol. 2011 (2011) 842849.
[32] Y. Zhang, C.L. Luo, B.C. He, J.M. Zhang, G. Cheng, X.H. Wu, Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response *in vitro*: a novel vaccine for renal cell carcinoma, Int. J. Oncol. 36 (2010) 133–140.
[33] L. Zitvogel, A. Regnault, A. Lozier, J. Wolfers, C. Flament, D. Tenza, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes, Nat. Med. 4 (1998) 594–600.