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A membrane vesicle-based dual vaccine against melanoma and Lewis lung carcinoma

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ABSTRACT

In the past few years, cell-derived membrane vesicle-based tumor vaccines have been considered as valuable new tools for cancer immunotherapy. Despite promising results in cancer clinical trails, an improved method is urgently needed for high efficiency tumor vaccines for a broad spectrum of tumors. Here we developed a single membrane vesicle-based vaccine, which is active in repressing both melanoma (B16) and Lewis lung carcinoma (LLC) tumor growth. By using the intrinsic function of dendritic cells in the processing and presentation of antigens, we generated dendritic cell (DC)-derived membrane vesicles (DC-mv) bearing tumor antigens from both B16 and LLC cells. Vaccination with this DC-mv-based dual vaccine induced specific cytotoxic T lymphocytes (CTL)-dependent tumor rejection and suppressed the growth of both types of tumor xenografts in mice. In addition, induction of CTL by this vaccine resulted in cross-protection responses and consequently enabled significant enhanced anti-tumor effects, indicating the synergistic anti-tumor activity. Our study suggests that the DC-mv-based vaccine holds great potential as a highly effective, versatile, cell-free vaccine for inhibition of multiple types of tumor growth.

1. Introduction

A tumor vaccine based on initiation of T-cell-mediated anti-tumor responses by recognition of tumor specific antigens is an attractive strategy for cancer immunotherapy [1–3]. In the past decade, development of cell-derived membrane vesicle-based vaccines for cancer immunotherapy has attracted considerable interest [4–6]. The major advantage is that nanometer-sized membrane vesicles have the molecular composition and biological functions that are not available from either discrete molecules or engineered nanomaterials [7–9]. It has been proved that exosomes, extracellularly secreted membrane vesicles, are naturally occurring minimal antigen-presenting units [10]. Previous studies have shown the potential for membrane vesicle-based vaccines in the initiation of tumor antigen-specific T-cell-mediated anti-tumor effects [11–13]. However, the newly emerging concept of a membrane vesicle-based tumor vaccine used for cancer immunotherapy is still not widely accepted. Further assessment of their feasibility as a therapeutic strategy for cross-protection and reduction or avoidance of the direct use of tumor cells is urgently needed.

Dendritic cell (DC)-derived exosomes (Dex) have generated considerable interest due to their potent anti-tumor immunity and minimal side effects both *in vitro* and *in vivo* [14,15]. Dex have been shown to contain all the necessary molecules required for the activation of specific T-cell responses [16]. It has been demonstrated that Dex derived from tumor antigen-pulsed DCs can induce cytotoxic T lymphocytes (CTL) priming and repress the growth of established murine tumors in a T-cell dependent manner [17,18]. To date, two phase I clinical trails of Dex in melanoma and non-small cell lung cancer patients have been completed [19,20]. Both studies demonstrated this therapeutic is safe and beneficial for cancer patients. Though obvious achievements have been confirmed in clinical trails, challenges remain in optimizing current Dex vaccines. One of the major challenges is that current Dex-based

Abbreviations: DC, dendritic cell; Dex, dendritic cell-derived exosomes; DC-mv, dendritic cell-derived membrane vesicles; Tex, tumor-derived exosomes; CTL, cytotoxic T lymphocyte; TEM, transmission electron microscopy; MVB, multivesicular body.

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vaccines do not prompt a sufficiently high enough anti-tumor response. In addition, the development of a single vaccine against a broad variety of tumor types remains challenging.

Previous studies have shown that exosomes transfer tumor antigens that exist in different tumors allowing T-cell-dependent cross-protection among different tumors in mice [21]. This result initially proved that protection against different tumors might be possible *via* exosomes containing a set of multiple tumor antigens. DC-derived membrane vesicles (DC-mv) are nanometer-sized vesicles generated by homogenization of DCs and ultracentrifugation [22]. These vesicles closely resemble Dex and share their ability to carry multiple cell-derived antigens [22,23]. We hypothesized that DC-mv may represent an antigen delivery vehicle that could be used as a highly efficacious membrane vesicle-based vaccine for cancer immunotherapy. The anti-tumor ability of DC-mv containing antigens from different types of tumors may have cross-protection and synergetic effects against these tumors.

In the present study, we established a dual vaccine based on DC-mv bearing antigens from two tumor cell lines, murine melanoma (B16) and murine Lewis lung carcinoma (LLC). B16 and LLC cells are both derived from C57BL/6J mouse and are commonly used as tumor models in anti-tumor studies [24,25]. We investigated the ability of DC-mv in the induction of T-cell-mediated anti-tumor responses *in vitro* and *in vivo*. In addition, we examined the anti-tumor efficiency of the dual vaccine (DC-mv$_{B16/LLC}$) against B16 and LLC tumor xenografts and made a comparison with DC-mv carrying antigens only from B16 cells (DC-mv$_{B16}$).

2. Materials and methods

2.1. Animals and cell lines

Male C57BL/6 mice, 6–8 weeks of age, were purchased from the Experimental Animal Center, Peking University (Beijing, China) and were allowed to acclimate for 1 week before experimental use. All mice were kept under normal condition with water and food given *ad libitum*. Mouse care and use were in compliance with the principles and procedures outlined by the animal welfare committee of Peking University. The murine melanoma (B16) cell line was obtained from ATCC (Manassas, USA). The murine Lewis lung carcinoma (LLC) cell line was kindly provided by Professor Guanghui Ma (Institute of Process Engineering, Chinese Academic of Science, Beijing, China).

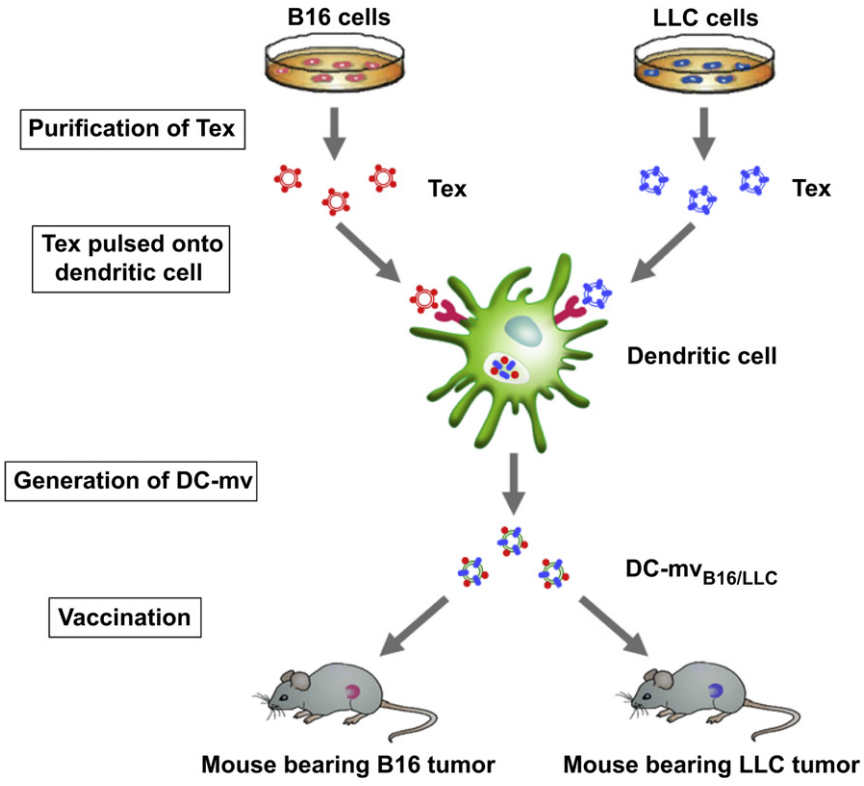
2.2. Generation of bone marrow-derived DC

Mouse bone marrow-derived DCs (BM-DC) were generated as previously described [26]. Briefly, bone marrow progenitor cells were collected from tibias and femurs of naïve mice. Non-adherent cells were removed by rinsing with phosphate buffered saline (PBS) after 4 h incubation at 37 °C in 5% CO_2. Adherent cells were then cultured in RPMI 1640 medium containing 500 U/mL IL-4 and 1000 U/mL GM-CSF (Peprotech, Rocky Hill, USA) to induce their differentiation to DCs. At day 7, immature DCs were obtained.

2.3. Preparation of Tex and DC-mv

Tumor-derived exosomes (Tex) were isolated from culture supernatants of B16 and LLC cells as described [27]. These Tex were used as tumor-derived antigens to challenge DCs. Briefly, B16 and LLC culture supernatants were first centrifuged at 800 g for 10 min at 4 °C to remove dead cells, and then centrifuged at 10,000 *g* for 30 min at 4 °C to remove cell debris. For the collection of exosomes, the supernatants were ultracentrifuged at 100,000 *g* for 70 min at 4 °C. The exosomes were washed once and resuspended in PBS and stored at −80 °C until use.

To obtain tumor antigen loaded DC-mv, 1×10^6 immature DCs were incubated with 5 μg B16 cell-derived exosomes for 6 h at 37 °C to generate DC-mv$_{B16}$; 1×10^6 immature DCs were incubated with the mixture of 5 μg B16 cell-derived exosomes and 5 μg LLC cell-derived exosomes for 6 h at 37 °C to generate DC-mv$_{B16/LLC}$. Control DC-mv isolated from immature DCs were designated DC-mv$_{blank}$. After two washes to remove free exosomes, cells were cultured for an additional 24 h in the exosome-free medium supplemented with 100 ng/mL lipopolysaccharide (LPS) for maturation. DC-mv were then isolated as previously described with minor modifications [23]. Briefly, DCs were collected and resuspended in PBS. The cell suspension was incubated for 10 min at 4 °C and transferred to a homogenizer, followed by 30 strokes. Immediately thereafter, this suspension was centrifuged successively at 800 g for 10 min, 10, 000 g for 30 min, and 100,000 *g* for 70 min at 4 °C. The pellets were resuspended in sterile 0.9% sodium chloride solution. The amount of protein in exosome and membrane vesicle preparations was measured by BCA protein assay (Applygen Technologies, Beijing, China). Scheme 1 summarizes the methods.



Scheme 1. Methods used to generate DC-mv$_{B16/LLC}$ dual vaccine and assess anti-tumor effect.