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Enhanced antitumor immunity by targeting dendritic cells with tumor cell lysate-loaded chitosan nanoparticles vaccine

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A B S T R A C T

Whole tumor cell lysates (TCL) have been implemented as tumor antigens for cancer vaccine development, although clinical outcomes of TCL-based antitumor immunotherapy remain unsatisfactory. In order to improve the efficacy of TCL-based vaccines, biomaterials have been employed to enhance antigen delivery and presentation. Here, we have developed chitosan nanoparticles (CTS NPs) with surface mannose (Man) moieties for specific dendritic cells (DCs) targeting (Man-CTS NPs). The Man-CTS NPs were then loaded with TCL generated from B16 melanoma cells (Man-CTS-TCL NPs) for *in vitro* and *in vivo* assessment. Potency of the Man-CTS-TCL NPs as cancer vaccine was also assessed *in vivo* by immunization of mice with Man-CTS-TCL NPs followed by re-challenge with B16 melanoma cell inoculation. We have shown here that Man-CTS-TCL NPs promote bone marrow-derived dendritic cells (BMDCs) maturation and antigen presentation *in vitro*. *In vivo* evaluation further demonstrated that the Man-CTS-TCL NPs were readily taken up by endogenous DCs within the draining lymph node (DLN) following subcutaneous administration accompanied by increasing in serum IFN-γ and IL-4 levels. Tumor growth was also significantly delayed in mice primed with Man-CTS-TCL NPs vaccine, attributable at least in part to cytotoxic T lymphocytes response. Moreover, Man-CTS-TCL NPs vaccine also exhibited therapeutic effects in mice with melanoma. Thus, we report here the Man-CTS-TCL NPs as effective anti-tumor vaccine for cancer immunotherapy.

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1. Introduction

Cancer immunotherapy is now considered a promising therapeutic approach against melanoma, leukemia, prostate and breast cancer [1–3]. The main purpose of this strategy is to prime naïve T cells and evoke long-term memory CD8^{+} T cells that attack tumor cells. Sufficient tumor antigen presentation, often by the antigen presenting cells (APCs) via the major histocompatibility complex (MHC) class I or II pathway, is critical in eliciting effective activation of both CD8^{+} and CD4^{+} T lymphocytes and thus determines the therapeutic efficacy of antitumor immunotherapy [4–6].

Dendritic cells (DCs) are professional antigen presenting cells (APCs) and are experts in priming T cells-mediated immunity [7,8]. DC-based vaccine has been extensively investigated as a feasible approach to enhance antigen-specific immune responses [6]. Most previous studies aimed at developing tumor vaccines that activate cytotoxic T lymphocytes (CTLs) responses and antibody secreting B cells through DCs mediated antigen presentation [9,10]. However,

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despite promising preliminary data, clinical outcomes of tumor vaccines have been disappointing [11,12]. One major challenge of the current antitumor immunotherapy is inefficient antigen delivery and subsequent induction of T cell mediated immune responses. In addition, the electrofusion process of DCs and tumor cells is time-consuming and laborious [13]. Moreover, external pulsing of DCs with selective peptides only result in limited stimulation of T cells due to the rapid turnover of class I peptides complex on the cell surface [14].

It has been recently shown that the use of whole tumor cell lysates preparation can overcome the above drawbacks and offers a comprehensive source of potential tumor antigens by inducing CTL responses and $CD4^+$ T helper cell activation [15,16]. Several clinical trials have used tumor cell lysates for *in vitro* DC priming [17,18]. However, soluble tumor lysates containing antigens and cytokines are inherently unstable and tend to result in poor DC uptake, inefficient antigen cross-presentation, and limited induction of CTL response [19]. Recent strategies for developing prophylactic or therapeutic vaccines have mainly focused on improving *in vivo* antigen delivery to specific DCs and prolonging their activation.

Biomaterial encapsulation of antigens has been proposed as a promising strategy to enhance immunogenicity during vaccination [20]. Not only could biomaterial encapsulation protect the antigens from degradation during *in vivo* administration, it also enables controlled release of antigens in a desired manner [21–24]. Moreover, surface of biomaterials could be modified with ligands or antibodies that are specifically recognized by DCs and used for DC targeting [25].

Chitosan is a cationic polysaccharide primarily derived from the exoskeletons of crustaceans and extensively used as vaccine delivery vehicles [21,26,27]. Earlier studies have reported chitosan-based nanoparticles, with particle size ranging from 100 to 500 nm, as feasible vehicles for *in vivo* delivery of proteins or peptides [28]. Moreover, chitosan nanoparticles have been reported to elicit significant adjuvant effect by stimulating innate immune responses [29]. Given the potential pro-inflammatory property of chitosan, we have developed chitosan nanoparticles with surface-decorated mannose (Man-CTS NPs) for specific DC targeting. Indeed, studies have shown that nanoparticles with surface-decorated mannose are effective delivery vehicles for APCs targeting [30–32]. Since the mannose receptor is expressed by immature dendritic cells, the mannose moiety on the surface of the Man-CTS NPs can be detected by DCs, via which antigen uptake would be enhanced [33,34]. Thus, in the present study, targeted delivery of Man-CTS-TCL NPs was assessed both *in vivo* and *in vitro*. In addition, to evaluate the potential of Man-CTS NPs as cancer vaccine delivery vehicle, we also encapsulated tumor cell lysates generated from B16 melanoma cells (Man-CTS-TCL NPs) and *in vivo* assessment of Man-CTS-TCL NPs on tumor prevention was performed.

2. Material and methods

2.1. Reagent and antibodies

Chitosan (CTS, Mw = 50,000, DD (degree of deacetylation) > 95%) was supplied by Ao'xing Biotechnology Co., Ltd. (Zhejiang, China), and used without further purification. Sodium alginate (ALG, viscosity: 160 mpa s, 20 °C, 1% aqueous solution) was supplied by Qingdao Crystal Rock Biology Development Co., Ltd. (Qingdao, China). Tetrabutylammonium (TBA) hydroxide and 4-Aminophenyl α-D-mannopyranoside ($MAN-NH_2$) were purchased from Aladdin (Shanghai, China). 2-chloro-1-methylpyridinium iodide (CMPI) was obtained from Alfa Aesar (Tianjin, China). Sodium sulfate, polysorbate 80 (Tween 80) and acetic acid were purchased from Sigma (St. Louis, MO). The MicroBCA™ Protein assay kit was supplied by Thermo Fisher Scientific Inc. (Rockford, IL USA). Phosphate Buffered Saline (PBS) (1×), RPMI-1640 medium, heat inactivated fetal bovine serum (FBS), trypsin EDTA 0.05%, penicillin/streptomycin (PEST) 10,000 Unit/mL/10,000 μg/mL, sodium pyruvate 100 mM, HEPES 1 M, 2-mercaptoethanol 50 mM, ACK lysing buffer and AlamarBlue® reagent were purchased from Life Technologies (Carlsbad, CA, USA). All the other chemicals were of analytical grade.

Anti-mouse ELISA kits IFN-γ, IL-4, IgG and IL-12p70 were purchased from eBioscience. Recombinant mouse GM-CSF and IL-4 were purchased from Peprotech (Rocky, Hill, USA). Fluorochrome-labeled anti-mouse monoclonal antibodies (CD3e, CD4, CD8a, CD80, CD86, MHCI, MHCII, CD11c, CD40 and CCR7) were purchased from eBioscience (CA, USA).

2.2. Cell lines and animals

Female C57BL/6 (6–8 weeks old) and female Balb/c mice (6–8 weeks old) were purchased from Academy of Military Medical Sciences (Beijing, China). All animal procedures were reviewed and ethically approved by Center of Tianjin Animal Experiment ethics committee and authority for animal protection (Approval No.: SYXK (Jin) 2011-0008). Mouse B16 melanoma tumor cell line [35] was purchased from the Cell Bank of China Academy of Sciences, and cultured according to the manufacture's guidelines.

2.3. Generation and isolation of tumor cell lysates

Tumor cell lysates (TCL) were generated as previously described [36]. Briefly, B16 melanoma tumor cell pellets were re-suspended in ice cold phosphate-buffered saline (PBS) at a 1×10^7/mL cell density and subjected to five freeze-thaw cycles of rapid freezing in liquid nitrogen (for 5 min) and thawing at 37 °C (for 5 min). The lysates were then centrifuged at 2000 g for 10 min to remove cellular debris. Proteins concentration was measured by BCA assay and the concentrated protein solution was diluted to appropriate concentration for *in vivo* and *in vitro* experiments.

2.4. Preparation of mannose-modified alginate (Man-ALG)

Tetrabutylammonium-alginate (ALG-TBA) was synthesized as previous report [37]. 500 mg (1.190 mmol) ALG-TBA was dissolved in 50 mL of anhydrous dimethylformamide (DMF). 152.1 mg CMPI (0.595 mmol) was added to activate the ALG-TBA in nitrogen at 0 °C. After 1 h, 4-Aminophenyl α-D-mannopyranoside (0.357 mmol) was added at room temperature and leave to react for 24 h. The reaction mixture was precipitated in absolute ethanol, repeatedly dissolved and precipitated for three times. The Man-ALG solution was transferred to a dialysis bag (MW = 3500 Da) to dialyze in distilled water for 2 days. The final product of Man-ALG was obtained after freeze-drying. The chemical structure of Man-ALG was confirmed by ^1H NMR spectroscopy (Varian Mercury 400, USA) and IR spectroscopy (Spectrum Instruments Co., Ltd. Brook Germany). The substitution degree (SD) of Man was defined as the ratio of reacted sugar unit to the total sugar unit of ALG.

2.5. Preparation and characterization of mannose-decorated chitosan nanoparticle encapsulation of tumor cell lysates (Man-CTS-TCL NPs)

Chitosan and tumor cell lysates (TCL) were dissolved in 1% acetic acid and ultrapure water, respectively. One mg/mL of chitosan solution and 1 mg/mL of TCL were obtained. The TCL solution was added drop-by-drop into chitosan solution and mixed at 1:1 (w/w). The mixture was then agitated at 300 rpm for 30 min to obtain

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