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ORIGINAL ARTICLE

Microbial HSP70 peptide epitope 407–426 as adjuvant in tumor-derived autophagosome vaccine therapy of mouse lung cancer

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Abstract Tumor-derived autophagome (DRibble) is an effective therapeutic cancer vaccine inducing T cell recognition and death of tumor cells in mice. However, the potential for improved anti-tumor response still remains. Our previous study demonstrated that two repeats of a mycobacterial $HSP70_{407–426}$ (M2) peptide acted as adjuvant in improving anti-tumor efficacy of human umbilical vein endothelial cell (HUVEC) vaccine. Here, a DRibble vaccine conjugated with M2 (DRibble-M2) was designed as a novel vaccine to enhance anti-tumor activity. Compared with DRibble alone, DRibble-M2 vaccination more significantly inhibited the growth of mouse Lewis lung cancer both in a subcutaneous tumor model and in a lung metastasis model. Higher expression of antigen-specific CTL was induced by DRibble-M2. DRibble-M2 induced higher CD83 and CD86 expression in DC2.4 and also improved the internalization of DRibble antigen into DC2.4. Our data indicated that DRibble-M2 is a potential vaccine for clinical cancer therapy.

Keywords Cancer immunotherapy · Autophagosome · $2mHSP70_{407–426}$ · Lung cancer

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Introduction

Lung cancer is a leading cause of cancer-related death in men and women. Most lung cancer patients are diagnosed at an advanced stage, and the overall 5-year survival rate is only about 15 % [1]. Despite surgical treatment, chemotherapy is the current first-line therapy that targets the heterogeneous signal transduction pathways. However, resistance to chemotherapy results in treatment failure [2–5]. Recent studies indicate that immunotherapy is an acceptable strategy for the treatment of lung cancer and represents an alternative clinical intervention [6, 7].

Adaptive immunity to tumors requires immunotherapeutic strategies to overcome the tolerance of tumor microenvironment and induce immunostimulation to train the immune system in recognizing tumor-associated antigens. Cancer vaccines are most popular immunotherapies developed for clinical anti-tumor therapy. Despite advances in cancer therapy, studies investigating well-defined tumor-associated antigens (TAA) are limited. However, minor antigens derived from tumor cells successfully trigger CD4 and CD8 T cell responses directly by inducing immune tolerance.

A majority of tumor antigens directly presented by the major histocompatibility complex (MHC) are derived from short-lived proteins (SLiPs) and defective ribosomal products (DRiPs) [8–10]. Tumor-derived autophagosome represents

cancer vaccine that inhibits tumor growth in multiple tumor models. DRibble is an autophagosome-enriched vaccine generated by tumor cell lines via inhibition of proteasomal and lysosomal functions. DRibble vaccine contains TAA, which is easily recognized by antigen-presenting cells (APCs) and elicits strong anti-tumor T cell response. Proteins including SLiPs and DRiPs are accumulated in stable double-membrane autophagosomal vesicles that are isolated through differential centrifugation [10].

Microbial HSP70 (mHSP) peptide 407–426 stimulates multiple inflammatory cytokines and enhances dendritic cell (DC) maturation. The $mHSP70_{407–426}$ binds with CD40 directly and triggers the CD40/CD40L signals on DC and induces T cell activation [11]. In our previous study, two tandem repeats of $mHSP70_{407–426}$ (M2) conjugated with glutaraldehyde-fixed human umbilical vein endothelial cell (HUVEC) efficiently inhibited tumor growth better than HUVEC vaccine alone by increasing the cytotoxic T cell (CTL) response in both prophylactic and therapeutic treatment in mouse models [12].

Herein, we report a therapeutic strategy that combines DRibble and M2 in a DRibble vaccine conjugated with M2 (DRibble-M2) vaccine, to induce effective T cell response against lung tumors. Using a mouse subcutaneous Lewis lung cancer model and lung metastasis model, we demonstrated that DRibble-M2 reduced tumor growth and metastasis better than DRibble alone. This combination vaccine enhanced the quantity and quality of antigen-specific CTL responses. Furthermore, antigen internalization experiments demonstrated that M2 enhanced DRibble endocytosis in DC. DRibble alone induces TLR2 and TLR4 signal activation, and DRibble-M2 combination promoted CD83 and CD86 expression on DC and induced DC maturation. This study explains the role of DRibble-M2 in lung cancer progression and offers a new strategy for treatment of established lung cancer and prevention of metastasis.

Materials and methods

Cell lines and mice

Mouse Lewis lung cancer cell line was maintained in RPMI 1640 (Lonza) supplemented with 10 % fetal bovine serum (Gibco), 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/mL streptomycin, and 100 mg/mL penicillin at 37 °C in 5 % CO_2.

DC2.4 mouse dendritic cell line, HEK293 TLR2, HEK293 TLR4 reporter lines, and control lines were maintained in DMEM (Lonza) supplemented with 10 % fetal bovine serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/mL streptomycin, and 100 mg/mL penicillin at 37 °C in 5 % CO_2.

Specific pathogen-free male C57BL/6 mice were provided by Jiangsu Experimental Animal Center (Yangzhou, Jiangsu Province, China) and housed in our laboratory under specific pathogen-free conditions. All mice were used in accordance with the protocol approved by the Animal Study Committee of China Pharmaceutical University.

Vaccine preparation

Cultured Lewis lung cancer cells were treated with 200 nmol/L bortezomib (Millennium) and 20 mmol/L ammonium chloride (Sigma Aldrich) for 24 to 48 h. DRibble was prepared as described previously [13]. Cells and debris were separated by centrifugation at 1600 rpm for 10 min. DRibble in suspension was collected by centrifugation at 12,000 rpm for 15 min. The total protein in DRibble was quantified by BCA protein quantification assay according to the manufacturer's protocol (Thermo Scientific). DRibble LC3 status was detected by Western blot, and autophagosome purity was measured by detecting the $LC3II^+$ frequency with flow cytometry. We generated the DRibble-M2 vaccine by directly mixing 1 mg/mL DRibble and 1 mg/mL M2 (synthesized by GL Biochemical Cooperation, Shanghai, China) in Dulbecco's phosphate-buffered saline (DPBS) (Lonza).

Tumor model and immunization procedure

C57BL/6 mice were randomly divided into four groups, with six animals each. Mice were treated with PBS (vehicle control), M2 (adjuvant control), DRibble (DRibble vaccine control), or DRibble-M2 (vaccine), respectively. Mice were vaccinated in the lower right flank with a subcutaneous injection (100 μg DRibble per mouse in 100 μL DPBS) 4 days after tumor challenge. Vaccine efficiency was measured in subcutaneous tumor and lung metastasis models.

In subcutaneous tumor model, all the C57BL/6 mice were injected with 5×10^5 Lewis lung cancer cells subcutaneously into the left flank. Tumor volume was measured every 2 days after tumor was inoculated for 10 days and calculated using the following formula: tumor volume = 0.52 × length × $width^2$. On day 23, mice from each group were euthanized and the tumor weight was measured.

In lung metastasis model, C57BL/6 mice were injected with 5×10^5 Lewis lung cancer cells intravenously. On day 28, mice from each group were euthanized. The lungs were harvested and fixed in Fekete's solution to count the number of metastases.

T lymphocyte proliferation

Three mice in each group were immunized with four types of vaccine and controls using the protocols indicated above. After 7 days, mice were sacrificed to isolate the spleen. The

spleens were harvested under sterile conditions and made into a single-cell suspension by grinding and passing through a 70-μm cell strainer (BD Bioscience). Erythrocytes were removed by density gradient using Lympholyte-M Cell Separation Media (Cedarlane). The splenocytes were resuspended in RPMI 1640 complete medium and seeded in 96-well plates (Costar) in triplicate at a concentration of 2×10^5/well. The spleen lymphocytes were incubated with and without 10 μg/mL of DRibble for 72 h, using 1.25 mg/mL ConA as a positive control and RPMI 1640 as the negative control. After stimulation for 72 h, all the supernatant in each well was discarded. Twenty microliters of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) was added to each well and incubated at 37 °C for an additional 4 h, followed by 100 μL dimethyl sulfoxide (Sigma-Aldrich) for solubilization of the product for 30 min at room temperature. The results were evaluated by measuring the absorbance at 570 nm using an ELISA reader (BioTek).

CTL assay

Splenocytes obtained from four groups were prepared as described in the proliferation assay. T cells were harvested as effectors by incubating them with target Lewis cancer cells in a 96-well plate in quadruplicate in effector/target ratios of 200:1, 100:1, 50:1, 25:1, and 12.5:1. Cytotoxicity was detected using a lactate dehydrogenase release assay according to the protocol described for CytoTox 96 kit (Promega). The cytotoxic effect was calculated using the following formula: $\text{cytotoxicity}\ (\%) = 100 \times (\text{OD}_{\text{experiment}} - \text{OD}_{\text{effector spontaneous}} - \text{OD}_{\text{target spontaneous}}) / (\text{OD}_{\text{target maximum}} - \text{OD}_{\text{target spontaneous}})$.

Flow cytometry

We incubated 5×10^5 DC2.4 cells with M2 (10 μg/mL), DRibble (10 μg/mL), and DRibble-M2 in a 24-well plate in triplicates, with DPBS as the negative control and LPS (10 ng/mL) as the positive control. After a 24-h stimulation, surface CD83 and CD86 expression was analyzed by LSRII flow cytometry (BD Bioscience). FACS data were collected with BD FACSDiva software and analyzed with TreeStar FlowJo software.

DRibble phagocytosis

Ten micrograms per milliliter of pH-sensitive cyanine dye CypHer5E (GE Healthcare) in PBS was used to stain 1 mg/mL of DRibble for 15 min at room temperature and washed twice with DPBS to remove the excessive free dye. One hundred micrograms per milliliter of stained DRibble was added to 5×10^5 DC2.4 cells in 1 mL of DMEM complete medium in triplicate on a 24-well plate. After a 24-h incubation, the fluorescence intensity and frequency of DRibble in DC2.4 were detected using LSRII flow cytometry (BD Bioscience).

Statistical analysis

Unpaired *t* test was performed using Prism (GraphPad Software). Data were presented as mean + SD. $P < 0.05$ suggested that the difference was statistically significant (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$).

Results

DRibble-M2 inhibits Lewis lung cancer growth in mice

DRibble alone was reported to induce anti-tumor activity in mice. To determine the degree of inhibition by DRibble-M2 vaccine against cancer growth, a subcutaneous growth Lewis lung cancer model in mice was used. DRibble was prepared and the LC3 status was assessed by immune blot. Compared with the healthy cell, the Lewis cancer cell-derived Dribble yielded a dramatically higher LC3II/LC3I ratio (Fig. 1a), and the LC3II^+ autophagosome was approximately 75.4 % based on flow cytometry (Fig. 1b).

The 4-day tumor-challenged C57BL/6 mice were treated with vaccines, and the tumor volume was measured

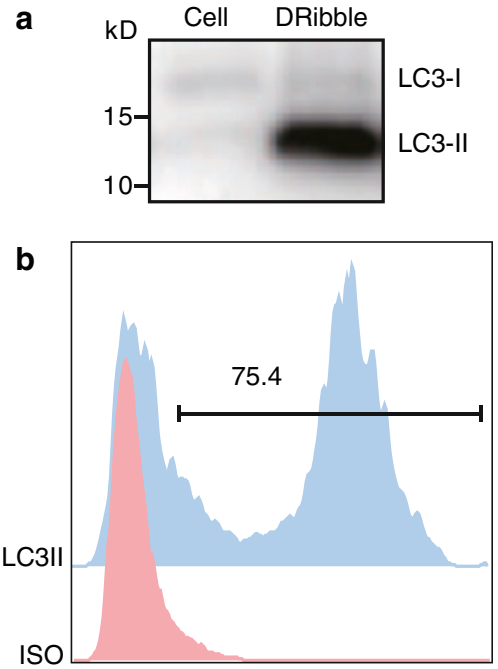


Fig. 1 Autophagosomes are enriched in DRibble vaccine. **a** LC3 status in DRibble derived from Lewis lung cancer cells was measured by western blot with anti-LC3B antibody; healthy Lewis lung cancer cells were used as control. **b** The frequency of LC3-II in DRibble was detected by flow cytometry analysis with an isotype antibody as control

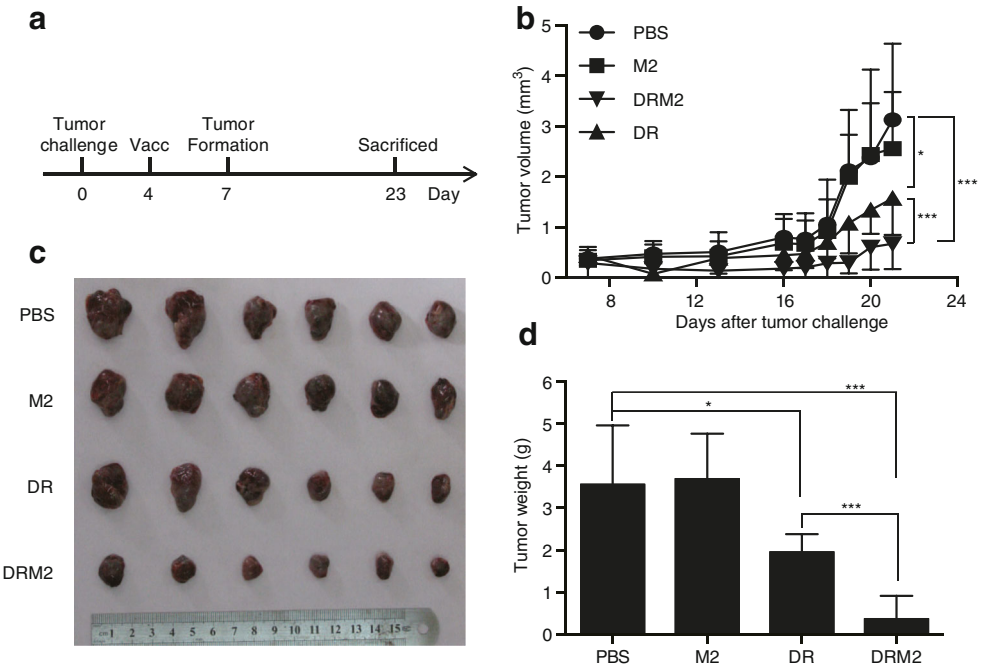


Fig. 2 Anti-tumor effects induced by DRibble-M2 in subcutaneous Lewis lung cancer-bearing mice. C57BL/6 mice were subcutaneously challenged with 5×10^5 Lewis lung cancer cells on day 0 and treated with PBS, M2, DRibble, and DRibble-M2 on day 4; mice were killed and tumors were excised on day 23 ($n = 6$). **a** Scheme of immunization procedure. **b** Tumor volume was examined with calipers every 2 days on day 7 post-tumor cell injection. **c** Tumors were excised and **d** weighed from mice immunized with PBS, M2, DRibble, and DRibble-M2

every 2 days since day 7 as shown in Fig. 2a. The tumor size was significantly inhibited in mice vaccinated with DRibble ($P < 0.01$) and DRibble-M2 ($P < 0.001$) compared with the PBS control group. No inhibition occurred in the M2 group. Tumor volume in the DRibble-M2 group was significantly suppressed compared with the DRibble alone group ($P < 0.001$) (Fig. 2b). Furthermore, mice were euthanized and solid tumor was excised on day 23. The DRibble-M2 vaccine dramatically reduced tumor growth in each group compared with PBS-treated ($P < 0.05$) and M2- treated ($P < 0.001$) groups, and DRibble-M2 vaccine worked more effectively than DRibble vaccine ($P < 0.001$) (Fig. 2c, d). DRibble-M2 vaccine attenuated tumor growth better than the other strategies in this study.

DRibble-M2 restrains tumor lung metastasis

To further demonstrate the therapeutic efficacy of DRibble-M2 vaccine, tumor formation rate was evaluated in a mouse model of lung metastasis. Tumor-bearing mice were immunized using the same strategy as subcutaneously injected tumor models. Four weeks after tumor challenge, mice were euthanized for evaluation of lung metastases. In contrast to the PBS-treated group, the DRibble vaccine-treated and DRibble-M2 vaccine-treated groups showed a dramatically significant reduction in lung metastases ($P < 0.01$ and $P < 0.001$, respectively). No significant difference was found between PBS and M2 groups. DRibble-M2 combination was more efficacious than DRibble alone ($P < 0.001$) (Fig. 3a, b). Similar results were obtained in the subcutaneous solid tumor model.

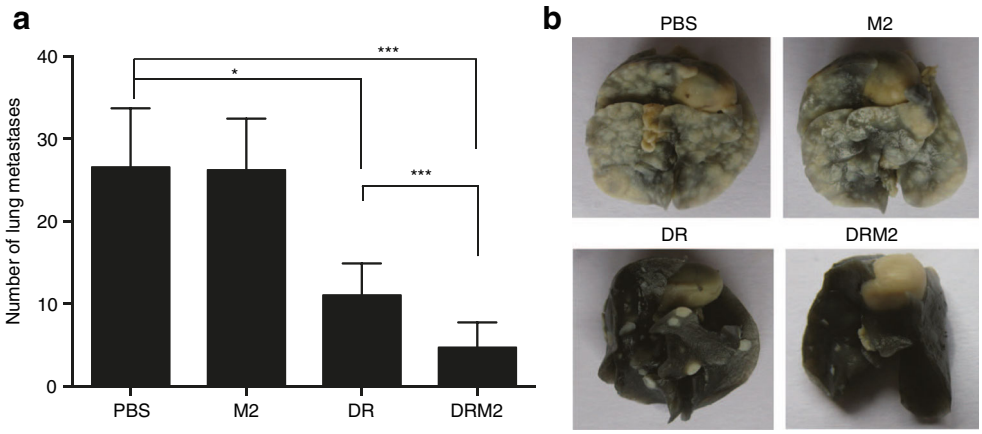
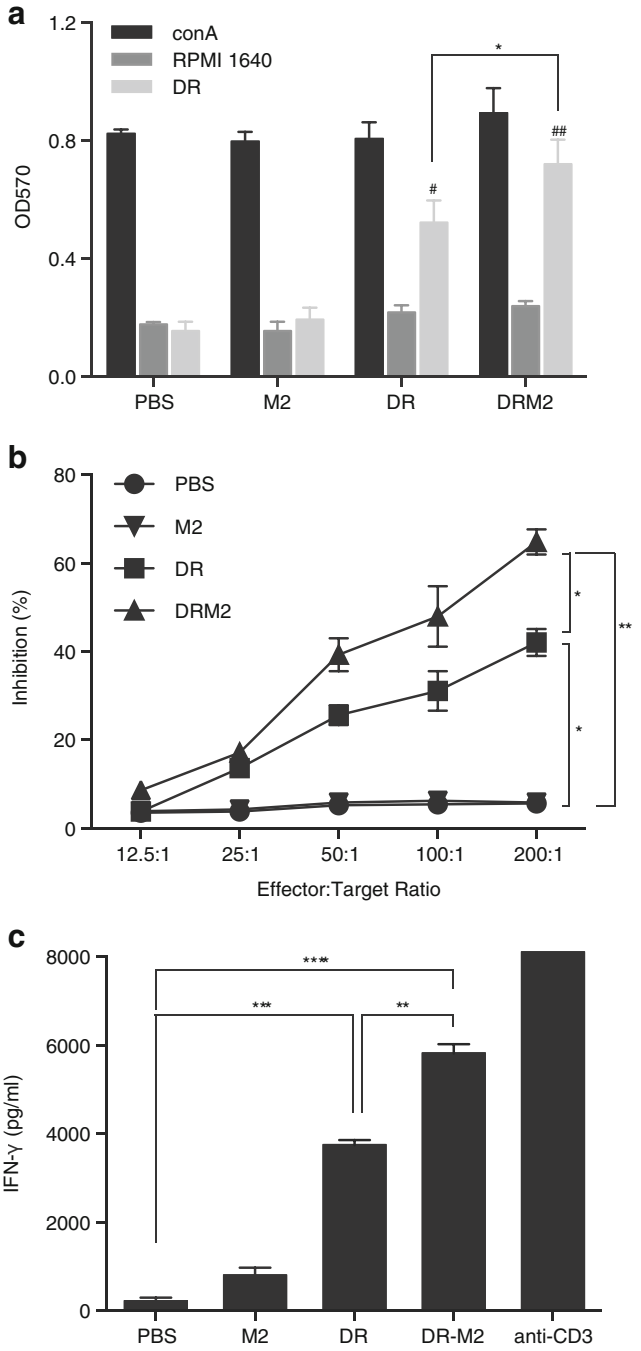


Fig. 3 DRibble-M2 inhibited lung metastases of Lewis lung cancer in mice. **a** C57BL/6 mice were subcutaneously challenged with 5×10^5 Lewis lung cancer cells on day 0 and treated with PBS, M2, DRibble, and DRibble-M2 on day 4; mice were killed and lungs were harvest on day 28 for counting the number of metastases ($n = 6$). **b** Macroscopic appearance of representative murine lungs flushed with India ink

Spleen lymphocyte proliferation

$mHSP70_{407–426}$ affected T cell activation. To study the role of M2 on lymphocyte proliferation, splenocytes from mice immunized with the four types of vaccine and controls indicated above were harvested and re-stimulated with DRibble. The lymphocyte proliferation was measured using an MTT assay. Compared with levels in PBS- and M2-immunized groups, lymphocytes from Dribble-injected ($P < 0.05$) and DRibble-M2-injected ($P < 0.01$) mice were greatly increased after re-stimulation with the DRibble antigen. Furthermore, T cell proliferation was higher in the DRibble-M2 group than in the DRibble group ($P < 0.05$) (Fig. 4a).



◀ **Fig. 4** T lymphocyte proliferation induced by DRibble-M2 vaccine. C57BL/6 mice ($n = 3$) were subcutaneously injected once with PBS, M2, DRibble, and DRibble-M2. **a** The spleens were harvested and T lymphocyte proliferative responses were detected with DRibble antigen in vitro; ConA and RPMI 1640 were used as positive and negative controls, respectively; $^{\#}P < 0.05$ compared with PBS; $^{\#\#}P < 0.01$ compared with PBS. **b** Spleen T lymphocytes from the mice immunized with indicated vaccine were cocultured with Lewis lung cancer cells at the ratio of effector/target for 200:1, 100:1, 50:1, 25:1, and 12.5:1. The killing frequency of target cells was measured by testing lactate dehydrogenase released in the cell culture supernatant. **c** Dribble-vaccinated spleens were harvested, activated in vitro with anti-CD3, and expanded with IL-2. The secretion of IFN-γ from Dribble-primed effector T cells was assayed by ELISA after stimulation with indicated antigens. Results are shown as the means + SD and representative of two or more independent experiments

CTL effect on Lewis cells

To further investigate the unique cellular immune response induced by DRibble-M2, antigen-re-stimulated T cells from immunized mouse spleens were tested as effector cells and incubated with target Lewis cells. PBS and M2 showed no lytic activity, and the frequency of target cell cytolysis was dramatically increased in the DRibble ($P < 0.05$) and DRibble-M2 ($P < 0.01$) groups. DRibble-M2 elicited stronger CTL response than DRibble alone ($P < 0.05$) (Fig. 4b). Dribble-treated mouse spleens were harvested, activated in vitro with anti-CD3, and expanded with IL-2. The secretion of IFN-γfrom the effector T cells was assayed after stimulation with different antigens. Dribble-primed effector T cells secreted antigen-specific IFN-γ when stimulated with both DRibble and DRibble-M2 antigens, rather than M2 alone. DRibble-M2 induced significantly higher Dribble-specific IFN-γ than DRibble vaccine ($P < 0.01$) (Fig. 4c).

DRibble-M2 amplifies DC activity

TLR ligands are usually used as adjuvants for immune therapy. Here, the TLR2 and TLR4 signal was evaluated with HEK293-TLR reporter cell lines by monitoring the levels of secreted embryonic alkaline phosphatase (SEAP) in culture supernatant. Vaccines in this study were incubated with HEK293 lines. The results showed that both DRibble and DRibble-M2 potentially triggered similar TLR2 and TLR4 activation. However, the signal in the M2 group was not detectable compared with the PBS group (Fig. 5).

M2 stimulated DC maturation by augmenting CD40/CD40L signal via direct binding with CD40 on APCs. Thus, the DC2.4 line was incubated with antigens and controls to determine the surface CD83 and CD86 expression by flow cytometry. DRibble and M2 alone slightly

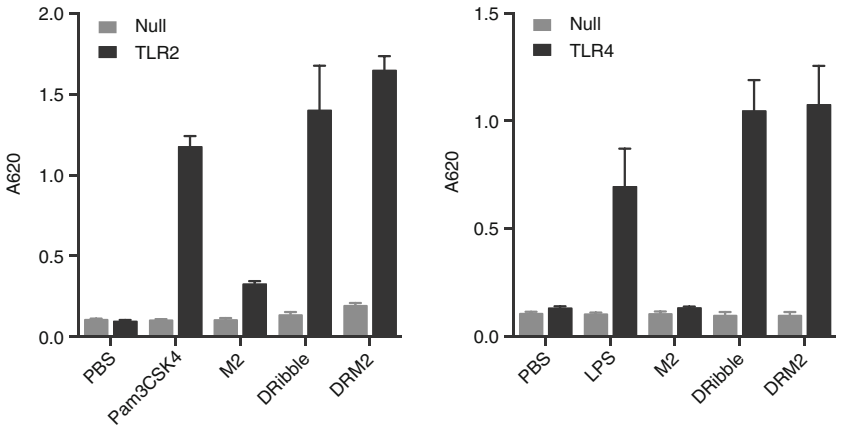


Fig. 5 DRibble-M2 induced TLR2/4 signals. HEK293 TLR2 and Null and HEK293 TLR4 and Null cells were incubated with PBS, 20 μg/mL M2, 20 μg/mL DRibble, 20 μg/mL DRibble-M2, 10 ng/mL Pam3CSK4, and 100 ng/mL LPS overnight. The supernatant SEAP production following TLR signal activation was evaluated by reading the optical density (OD) at 620 nm according to QUANTI-Blue Detection assays. Results are shown as the means + SD and representative of two or more independent experiments

increased the CD83 and CD86 expression, respectively, on DC2.4, when compared with PBS. DRibble-M2 vaccine elicited high CD83 and CD86 production similar to LPS (Fig. 6).

Our findings indicated that M2 improved T cell induction capacity of DRibble antigen. DRibble alone acted as TLR2 and TLR4 agonists and, in combination with M2, induced DC maturation, to trigger a stronger anti-tumor response compared with either antigen alone.

DRibble-M2 internalized in DC2.4

Antigen internalization in APC is important for antigen presentation. DRibble antigen is an autophagosome derived from cancer cells and contains multiple autophagy substrates to facilitate antigen processing in APC. DRibble endocytosis was monitored by labeling with pH-sensitive dye CypeHer5E, which is minimally fluorescent at neutral pH, but highly fluorescent when DRibble is fused with acidic cell proteasomes or lysosomes [14]. CypeHer5E-labeled DRibble was incubated with DC2.4 cells overnight. Under fluorescence microscope, DRibble was fused with lysosome and distributed around the nucleus in the cells (Fig. 7a). By measuring the frequency of endocytosis of DRibble, DC2.4 was obtained for flow cytometry. CypeHer5E signal was detected in 49 % of DC2.4 cells in the DRibble group, and the signal in DRibble-M2 group was increased to 58 % (Fig. 7b). The results indicated that M2 increased antigen internalization frequency for effective antigen presentation.

Toxicity determination

In order to examine vaccine safety, mice were injected with DRibble-M2 and monitored daily for possible adverse effects. All the mice acted normally similar to untreated healthy mice and showed no changes in body weight, habits, appearance, or life span. Further, heart, liver, spleen, lung, and kidney showed no pathologic changes under microscopic analysis (Fig. 8).

Discussion

Cancer immunotherapy has been designated as a breakthrough in cancer treatment. It is a well-deserved recognition

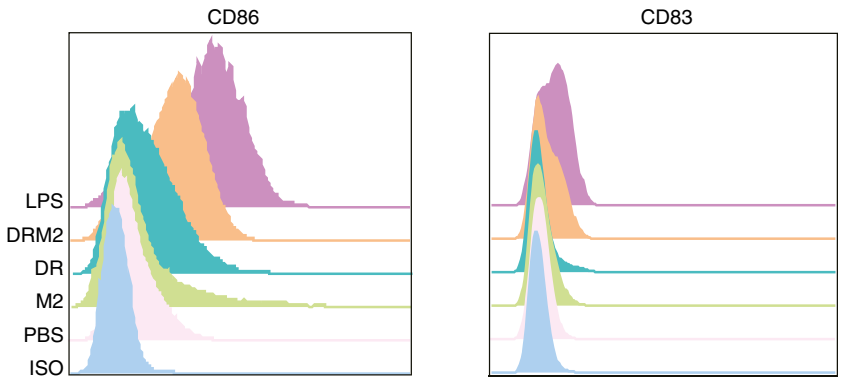


Fig. 6 DRibble-M2 promoted DC maturation. DC2.4 were overnight incubated with PBS, 100 μg/mL DRibble, 100 μg/mL DRibble-M2, 100 μg/mL M2, and 100 ng/mL LPS. **a** CD86 and **b** CD83 expression on DC2.4 was identified by flow cytometry. Results are representative of two or more independent experiments.

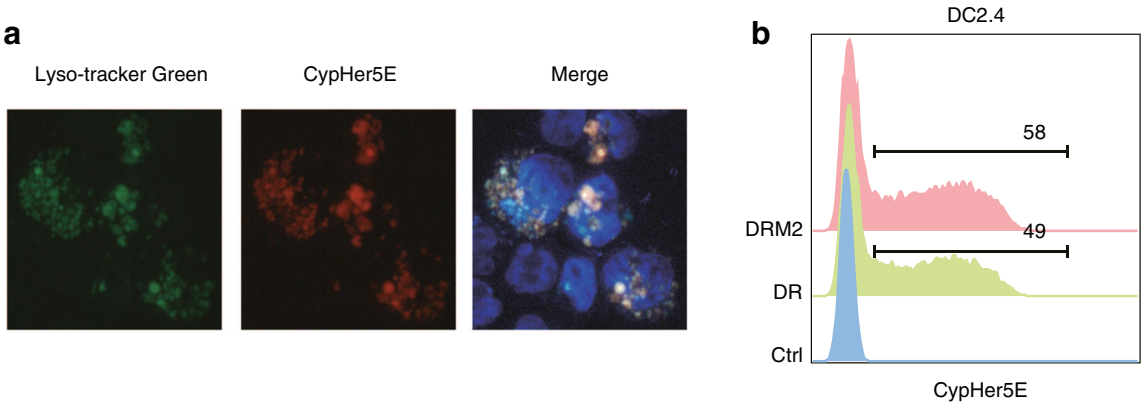


Fig. 7 Internalization of DRibble-M2 in DC2.4 cells. DC2.4 cells were treated with PBS, CypHer5E-labeled DRibble (20 μg/mL), and DRibble-M2 (20 μg/mL) overnight. **a** CypHer5E-labeled DRibble was co-located mostly with the lysosomes in cells by fluorescent image (×200 original magnifications). **b** The efficiency of DRibble internalization in DC2.4 was detected for CypHer5E signal by flow cytometry

of tumor immunologists who dedicated decades of hard work in deciphering the key roles played by the immune system in carcinogenesis as well as cancer therapy. The work on checkpoint inhibitors such as anti-CTLA4 and anti-PD1 was recognized in a subset of patients in clinical trials over the past 5 years. With the increased recognition of the significance of immune system in cancer clinical outcomes, immunotherapy has proven effective as an effective anti-cancer and anti-virus treatment [15–17].

Vaccines play an important role in immune response against a wide range of antigenic determinants that may be unique to individuals or dependent on their unique T cell receptor (TCR) repertoire. The majority of peptides presented on tumor MHC molecules are derived from rapidly degraded SLiPs and DRiPs, in addition to overexpressed proteins and neo-epitopes following mutations. They are likely to be dominant epitopes presented by MHCs in tumor cells. However, the immune system has been poorly educated to recognize them, since SLiPs and DRiPs are scarcely available for cross-presentation by professional APC [18, 19]. An autophagosome-based vaccine containing SLiPs and DRiPs as well as danger signals and agonists of TLR2 and TLR4 examined in this study represents a potential immune activator in multiple types of cancer. It facilitates assessment of the magnitude of therapy-induced T cell responses and serves as a vaccine for cancer treatment [20–22]. Studies involving mouse models show successful cross-presentation of antigens in DRibble leading to dramatic anti-tumor effects. Despite the availability of checkpoint inhibitors, multiple immune activators such as TLR agonists and inorganic adjuvant act as adjuvants when used as combination therapies. Our previous study indicates that DRibble vaccine inhibits the growth of murine lung cancer as well as other types of solid tumors by inducing a strong antigen-specific T cell response, while conjugates of DRibble with alpha-alumina nanoparticles increased the anti-tumor response dramatically. Further, the addition of the OX40 antibody prevents lung metastases in 3LL lung tumors of mouse [23].

In this study, a bioactive peptide was introduced to enhance anti-tumor activity. mHSP70 acts as a chaperone to present external proteins to MHC-II and cross-present them to MHC-I pathways. $mHSP70_{407–426}$ is an immunoactive epitope that enhances DC functions and induces Th1 and $CD8^+$ T cell response. In our early study,

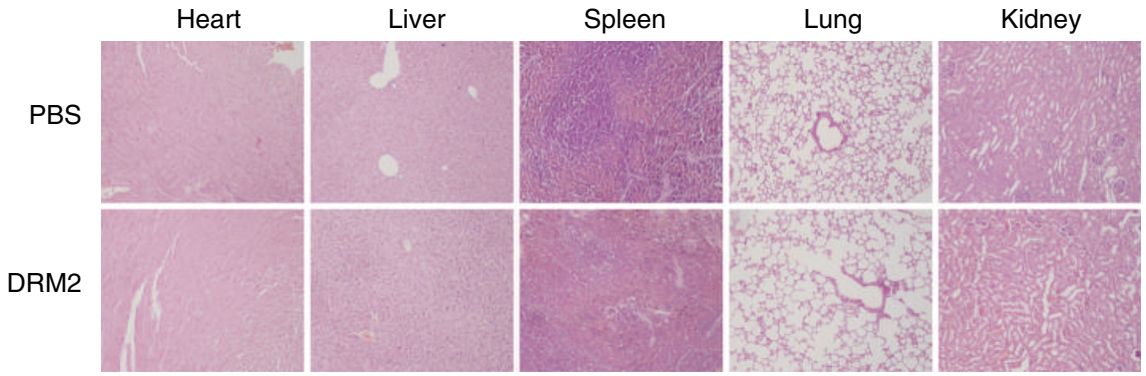


Fig. 8 General observation of DRibble-M2 toxicity in mice. H&E staining (×100 original magnification) of the heart, liver, spleen, lung, and kidney in DRibble-M2-immunized mice. Compared with untreated mice, no noteworthy pathologic changes were observed

two tandem repeats of $mHSP70_{407–426}$ designed as adjuvants improved immunity and triggered a robust anti-tumor response [24]. M2 conjugated with glutaraldehyde-fixed HUVEC as a vaccine targeting vasculature inhibits murine H22 hepatocellular carcinoma growth by inducing strong T cell response to endothelial cells better than HUVEC alone [12]. Hence, we combined M2 with DRibble vaccine in an effort to reverse the immune suppression in tumor-bearing mice using a Lewis mouse lung cancer model. In our study, we found M2 combined with DRibble vaccine inhibiting tumor growth in mice and also preventing tumor metastasis in lungs better than using DRibble alone. In combination with M2, antigen-specific T cells were significantly induced by DRibble in C57BL/6 mice when compared with DRibble alone. DRibble is transported in cells via the endoplasmic reticulum-associated degradation (ERAD) pathway. It combines with lysosome for degradation. Interestingly, by tracking DRibble with fluorescent imaging and flow cytometry, we found that M2 facilitated DC-induced endocytosis of DRibble, which was essential to trigger immunogenic response and augment cross-presentation of DRibble antigens. DRibble comprises damage-associated molecular pattern molecules (DAMP), including HMGB1, RNA, dsDNA, HSP, and calreticulin [10, 25]. Here, we demonstrated that DRibble derived from Lewis lung cancer cells induces murine TLR2 and TLR4 signaling. Additionally, DRibble-M2 combination dramatically increased CD86 and CD83 expression on DC2.4 and enhanced DC maturation better than DRibble or M2 alone. Our findings strongly confirmed that DRibble in combination with M2 induced robust anti-tumor response against established Lewis lung cancer.

DRibble expressed in different cancer cells has been shown to upregulate genes. DRibble derived from mouse sarcoma not only elicited immune response to homologous tumors but also cross-reacted with different types of sarcoma and cross-protected vaccinated hosts against non-homologous tumor challenge [10]. From a translational point of view, autophagosomes may be a potential source of anti-tumor vaccines that contain common tumor antigen-enriched endogenous alarmins. They can be appropriately modified and adapted to enhance immune responses.

In conclusion, M2 represents an adjuvant, which when combined with autophagosomes yields a DRibble-M2 vaccine. Such conjugated vaccines facilitate the generation of tumor-specific and tumor-associated antigens to induce robust anti-tumor immune responses. DRibble-M2 significantly enhanced the inhibition of tumor growth better than DRibble vaccine alone. It induced potent T cell response by improving the antigen-presenting capacity of DC to T cells. DRibble-M2 vaccine might be used to augment immunity in clinical applications involving anti-tumor immunotherapy.

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Compliance with ethical standards

Conflicts of interest None

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