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A simple and powerful co-delivery system based on pH-responsive metal-organic frameworks for enhanced cancer immunotherapy

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A B S T R A C T

Tumor-associated antigens (TAAs)-loaded nanoparticles are able to be actively internalized by antigen-presenting cells (APCs) and have shown promising potential in cancer immunotherapy. However, current TAAs delivery strategy exhibits limitations of complicated synthesis process, low loading efficiency and inefficient $CD8^+$ cytotoxic T lymphocyte activation leading to unsatisfactory therapeutic effect. Thus, the construction of novel TAAs-delivery systems for enhanced cancer therapy is highly desirable. In this work, we fabricated a very simple yet powerful antigens-delivery system for cancer immunotherapy based-on pH-responsive metal-organic frameworks (MOFs) with size about 30 nm. TAAs can be loaded into MOFs in the one-pot synthesis process and released with the degradation of MOFs in the acidic environment of endo/lysosome as the result of relatively labile metal-ligand bonds. The endosomolytic nanoparticles would facilitate protein antigens escape from endo/lysosome and optimal for enhancing antigen cross-presentation. Furthermore, the introduction of immunostimulatory unmethylated cytosine-phosphate-guanine oligonucleotide (CpG) through Watson–Crick base pairing would further enhance $CD8^+$ cytotoxic T lymphocyte responses. We demonstrated that the method to co-delivery antigens and immunostimulatory molecules was very simple, convenient and effective and showed no obvious toxicity both in *vitro* and in *vivo*. This method gave a high antigens-loading capacity and the maximal antigen encapsulating efficiency was about 55% (w/w). Additionally, the pH-responsive co-delivery system exerted enhanced antitumor outcome (about 100% survival) in B16-OVA melanoma cancers in *vivo*. Furthermore, we confirmed that this high rating of therapeutic effect was attributed to the recruitment of tumor-killing immunocyte. This work demonstrates the ability of pH-responsive, endosomolytic MOFs to induce strong cellular immune responses for cancer therapy by co-delivery of CpG ODN and antigens.

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1. Introduction

Immunotherapy has been intensively studied over the past several years and shows great promise for cancer treatment. Cancer immunotherapy primarily focuses on stimulating individual's own immune system to recognize and kill cancer cells. Up to now, various approaches have been developed for cancer immunotherapy, including the administration of tumor-associated antigens (TAAs), with or without adjuvants [1]. However, TAAs have poor immunogenicity and insufficient tumor antigen uptake by antigen-presenting cells (APCs), which leads to undesirable cellular and humoral immunity [2,3]. To overcome these problems, much effort has been made to develop novel therapeutic cancer vaccines strategies that can persistently activate cellular and humoral immunity to combat existing tumors. Therein, a key consideration is how to efficiently deliver TAAs to APCs and maximize cross-presentation for enhancing $CD8^+$ cytotoxic T lymphocyte (CTL) responses [4]. To this end, biocompatible nanoparticles-based antigen-delivery systems have been developed recently, as they were shown to effectively protect antigen against degradation and facilitate uptake by professional APCs [5,6]. For instance, liposomes, polymer-based nanoparticles and inorganic nanoparticles, have been applied as

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adjuvants to enhance vaccine efficiency [7–10]. However, these nanomaterials generally lack intrinsic immune-stimulatory effects, which limits the further improvement of immune efficiency [11–13]. Researchers have also attempted to co-deliver antigens and immunologic adjuvant for improving vaccine-induced immune responses [14,15]. However, most of these reported works need laborious preparation procedures and show low loading efficiency which limits their widespread use. More importantly, the cellular uptake of nanoparticle-based adjuvants along with loaded antigens occurs by an endo/lysosomal processing pathway, resulting in the generation of $CD4^+$ helper T cell responses and the minimal CTL stimulation. Thus, an easy-to-fabricate nanovaccine that has high antigens loading capacity and facilitates antigens escape from endo/lysosomal to enhance cross-presentation is still lacking and remains highly desirable in cancer immunotherapy.

Since Ferey and colleagues firstly suggested that Materials of Institute Lavoisier (MIL) family could be used as drug delivery carrier [16], metal-organic frameworks (MOFs) have been vastly studied as ideal drug delivery materials owing to their high loading capacity, chemical and structural diversity, low cost and biodegradability [17]. To date, different therapeutic agents have been developed to incorporate into different MOFs for drug delivery [18–20]. However, these guest molecules are mostly small molecules and especially, minimal attention has been paid to biomolecules such as proteins, nucleic acid, peptides, etc. [21–23]. In this sense, we envision that proper designing the open architectures and pore volumes of MOFs would provide unprecedented opportunities for biomacromolecule antigen delivery and cancer immunotherapy, which however has remained unexplored so far.

In this work, by utilizing lanthanide ions and guanine monophosphate (GMP) as MOF coordinating partners, we developed a simple yet powerful co-delivery system for high-performance cancer immunotherapy. By taking advantage of the tailorability of MOF materials, a model vaccine ovalbumin (OVA), the most completely characterized antigen for studying antigen presentation in *vitro* and in *vivo*, is used as a dopant and directly incorporated into the frameworks during the synthetic process. Moreover, GMP is a DNA nucleotide and can enhance the binding capability of CpG to the GMP/Eu MOF surface through Watson–Crick base pairing, which is convenient for co-delivering antigens and immunostimulator. Importantly, the coordination of Eu and GMP dissociates under pH 5.0, which facilitates protein vaccines escape from endo/lysosome (pH 4–5) into the cytosol [24–26], optimal for enhancing antigen cross-presentation. More importantly, CpG is an endosomal-acting oligonucleotide adjuvant and will be released in the endo/lysosome, leading to the improvement of immune responses. Therefore, we envision that this easy-to-fabricate and pH-responsive endosomolytic nanovaccine would provide an advanced platform for promoting a Th1-type immune response and anticancer therapeutic efficiency.

2. Materials and methods

2.1. Materials

OVA and GMP were purchased from Sigma-Aldrich (USA). Fluorescein isothiocyanate (FITC) and europium chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) were obtained from Aladdin. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen. Purified anti-mouse TNF-α, biotin conjugated anti-mouse TNF-α cocktail, mouse TNF-α recombinant protein, anti-mouse IFN-γ, biotin anti-mouse IFN-γ, mouse IFN-γ recombinant protein, anti-Mouse IgG biotin, anti-Mouse IgG2a biotin, Anti-Mouse IgG1 biotin, and avidin-HRP were purchased from eBioscience. CpG oligodeoxynucleotides (5′-TCCATGACGTTCCTGACGTT-3′), 6-carboxytetramethylrhodamine (Tamra)-labeled CpG and control DNA (5′-AAAAAAAAAAAAAAAAAAAA-3′) were synthesized by Sangon Biotechnology Inc. (Shanghai, China). The mouse leukemic monocyte macrophage cell line (RAW264.7 cell line) was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai). C57BL/6j mouse was achieved from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. (Beijing, China).

2.2. Characterizations

The morphology of samples was observed using a scanning electron microscopy (SEM, Model JSM-7500F, Japan). Zeta potentials (ζ-potential) were estimated on a Zeta Potential/BI-90Plus particle size analyzer (Brookhaven, USA). FT-IR spectra were recorded with a Spectrum One FT-IR spectrometer (Perkin Elmer Instruments Co. Ltd., USA). UV–Vis absorption spectra were recorded using a UV-2700 (Shimadzu, Japan). Fluorescence measurements were carried out on F-7000 spectro fluorometer (Hitachi, Japan).

2.3. OVA loading and release behaviors

To prepare OVA-loaded GMP/Eu complex and optimize drug loading efficiency, we first synthetized FITC-labeled OVA owing to the interference of the UV-absorption peak of GMP (260 nm) on OVA (280 nm). FITC-labeled OVA was synthesized by incubating 100 mg OVA with 5 mg FITC for 1 h at room temperature in 0.1 M carbonate buffer (pH 9.2). The mixture was slightly stirred in the dark at 4 °C for 18 h. Unreacted FITC were removed by dialysis. Then we prepared OVA-loaded GMP/Eu complex (MOF-OVA) as follows:

GMP (1 mL) and OVA-FITC (2 mL) or OVA were dissolved in pure water (18 mL) under the different concentrations of OVA. Then $EuCl_3$ solution (400 μL) was added to the mixture under slight stir. Solid products appeared immediately. The reaction was kept for 5 min at room temperature, then collected by centrifugation at 8000 rpm for 5 min and washed thoroughly with pure water.

Antigens loading efficiency (ALE) was calculated according to the following formulae: ALE (%) = (weight of loaded OVA/weight of MOF-OVA) × 100.

In *vitro* release OVA from MOF-OVA was studied in PBS buffer (pH 7.4 and 5.0) at 37 °C. Two copies of materials (8 mg) were placed in 4 mL of PBS (pH 7.4 or 5.0) at 37 °C under stirring. At desired time intervals, 400 μL solutions were taken out and replenished with an equal volume PBS. Quantum antigens concentrations were determined by bicinchoninic acid (BCA) assay according to the protocol manual.

2.4. pH-responsive nanoparticles degradation process

In *vitro* degradation behaviors of MOFs were studied by immersing corresponding powders (2 mg/mL) in different buffer solution (pH = 7.4 and 5.0). The change of morphology was observed by SEM at different times (0, 3, h and 9 h). To further confirm the pH-responsive nanoparticles degradation process, time curves of the released GMP were carried out by using a UV–Vis spectrophotometry at 280 nm with different pH.

2.5. The loading and release behaviors of CpG

MOF-OVA (2 mg) materials were suspended in PBS (1 mL) and then 80 μL Tamra-labeled CpG with different concentrations (2, 1, 0.5, 0.25, 0.1 and 0.05 μM) were added into the solutions and stirred at room temperature for 2 h. The supernatant was collected by centrifugation at 8000 rpm for 5 min and analyzed by UV–Vis

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