Journal of Controlled Release 273 (2018) 1–12

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

An indoleamine 2, 3-dioxygenase siRNA nanoparticle-coated and Trp2-displayed recombinant yeast vaccine inhibits melanoma tumor growth in mice

Dong-qun Liu[a,b,1], Shuai Lu[a,1], Ling-xiao Zhang[a,b], Mei Ji[a,b], Shu-ying Liu[a], Shao-wei Wang[a], Rui-tian Liu[a,*]

[a] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Indoleamine 2, 3-dioxygenase (IDO)
Small interfering RNA (siRNA)
Nanoparticle
Tyrosinase-related protein 2 (Trp2)
Saccharomyces cerevisiae
Melanoma tumor

ABSTRACT

Therapeutic vaccine is a promising approach in cancer therapy. But tumor-associated antigen peptides have weak immunogenicity and cancer patients are often characterized by immunosuppression and tolerance, leading to less efficiency of immunotherapy. We here successfully developed indoleamine 2, 3-dioxygenase (IDO) siRNA nanoparticle-coated and tyrosinase-related protein 2 (Trp2)-displayed recombinant *Saccharomyces cerevisiae* (YCP). YCPs had positive charges with a diameter of approximately 5 μm, resulting in selective phagocytosis by APC cells. YCP-delivered siRNA and Trp2 successfully escaped from phagosomes, efficiently inhibited IDO expression in DCs, promoted the immune reaction of T cell against Trp2, increased the secretion of proinflammatory cytokines such as IFN-γ,TNF-α, and IL-6, and decreased the generation of regulatory T cells. Moreover, YCPs significantly inhibited melanoma tumor growth by alleviating immune tolerance and promoting Trp2-specific $CD8^+$ T cell immune response. These results suggest that *Saccharomyces cerevisiae* as a combined immunotherapeutic platform to simultaneously delivery IDO-siRNA and Trp2 epitope peptide is a promising vaccine system for melanoma treatment.

1. Introduction

Therapeutic cancer vaccines can induce protective immune response against cancer invasion, metastasis or recurrence, which rely on the antigen properties and the ability of antigen-presenting cells (APCs), especially dendritic cells (DCs), to present administered tumor antigens *via* the major histocompatibility complex (MHC) class I or II to $CD8^+$ or $CD4^+$ T cells, respectively [1]. DCs possess high functional plasticity in response to external stimuli, which allows them to orchestrate adaptive immune responses through generating proinflammatory or suppressive DCs. Therefore, increasing tumor antigen immunogenicity and selective delivery to DCs, promoting T cell immune responses, and preventing immune tolerance are attractive approaches to induce or enhance antitumor immunity.

Some microorganisms can be naturally recognized by APCs and act as vaccine adjuvants. However, direct administration of pathogen particles has potential risks [2]. One novel promising approach is to use of recombinant *Saccharomyces cerevisiae* which expresses antigen in the cytosol [3,4] or on the cell wall [5,6]. *S. cerevisiae* is a particularly attractive vaccine carrier, featuring targeted delivery of antigens to APC, inherent nonpathogen and adjuvant function [7,8]. *S. cerevisiae* has complex cell wall components and perfect natural size, inducing specific uptake by phagocytosis cells such as DCs and macrophages rather than most non-phagocytic cells, and avoiding the risk of antigen uptake and presentation by non-APCs [9]. Moreover, DCs recognize *S. cerevisiae* through dectin, mannose-fucose receptors and toll-like receptors (TLRs) such as TLR-2 and TLR-4, inducing maturation of dendritic cells (DCs) and the secretion of Th1-type cytokines through the pathogen-associated molecular patterns (PAMPs) [10–12]. These characteristics make *S. cerevisiae* a potential vaccine vehicle for cancer and infectious diseases. Unfortunately, *S. cerevisiae* promoted the generation of certain number of functional regulatory T cells by inducing the expression of indoleamine 2, 3-dioxygenase (IDO) in DCs [13,20]. It was reported that IDO can modulate adaptive immune responses by promoting immune-suppression and tolerance [14,15]. However, silencing of IDO mRNA and subsequent protein in DCs by small interfering RNA (siRNA)

* Corresponding author at: State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Haidian District, Beijing 100190, China.
E-mail address: rtliu@ipe.ac.cn (R.-t. Liu).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.jconrel.2018.01.013
Received 22 August 2017; Received in revised form 15 December 2017; Accepted 16 January 2018

reversed immunosuppression, preventing antigen-presentation DCs from an immunostimulatory to an immunosuppressive cell type [16,17].

Delivering siRNA to specific cells confronts a serious challenge. siRNA should be protected from degradation by endonucleases, aggregation with serum proteins, and the kidney filtration [18,19]. Many materials such as cationic lipid, polyethylene glycol-lipid, dioleoyl phosphatidylethanolamine were reported to formulate siRNA [20]. Polyethylenimine (PEI) is a synthetic polymer capable of forming non-covalent complexes with siRNA and protecting them from nucleolytic degradation. PEI-siRNA complexes can be efficiently taken up *via* endocytosis and escape from endosomes in the cytoplasm through the "proton sponge effect" *via* the repeating ethyleneamine groups and high charge density of PEI [21–23]. However, single immunotherapy such as providing exogenous antigen stimuli, expanding anti-tumor reactive T cells and antagonizing immune tolerance regulatory pathways often show less efficiency, combination therapy has been recommended for the treatment of cancer due to additive or synergistic anticancer activity [26–28]. To obtain an effective cancer vaccine, Herein, we developed a novel composite immunotherapeutic agent, YCP, using *S. cerevisiae* as a vaccine carrier which expressed melanoma tumor-associated antigen Trp2 epitope on the cell wall and was coated with cationic polyethylenimine (PEI)-IDO siRNA nanoparticles to overcome immune tolerance (Fig. 1). Compared with the previous siRNA delivery systems that lack specific and effective *in vivo* delivery methods to reduce the potential effectiveness of siRNA-based therapies [24,25], our strategy induced very specific siRNA uptake by APCs and educated APCs towards immunostimulatory phenotype to elicit strong antitumor immune response, resulting in greatly reduced amount of administrated siRNA and lowered toxicity to the major organs in body.

2. Materials and methods

2.1. Materials

Branched PEI-25k were obtained from sigma-Aldrich (St. Louis, MO, USA). Brefeldin A solution and following fluorescence-labeled antibodies including mouse PE/Cy7-anti-CD3 antibody, FITC-anti-CD4 antibody, FITC-anti-CD8a antibody, Percp-anti-CD8a antibody, PE-anti-IL4 antibody and APC-anti-IFN-γ antibody were purchased from Biolegend (San Diego, CA). LysoTracker® Red DND-99, RPMI medium 1640 basic, fetal bovine serum, penicillin streptomycin were purchased from Thermo Fisher Scientific (MA, USA). Recombinant mouse granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 were purchased from PeproTech (Rocky Hill, USA).

2.2. Peptide design and synthesis

The peptide H-SVYDFFVWL-OH, amino acids 180–188 from tyrosinase-related protein 2 (Trp2), was synthesized as an acetate powder by Beijing Protein Innovation Co., Ltd. (Beijing, China). The peptide contained a Trp2 epitope restricted by both human HLA-A2 and the murine major histocompatibility complex (MHC) class I molecule H-2K^b on B16 tumor cells. The disodium salt of Trp2 peptide was formulated as described previously [24] and their aliquots in 10 mM HEPES (pH 7.2) were stored at − 80 °C until use.

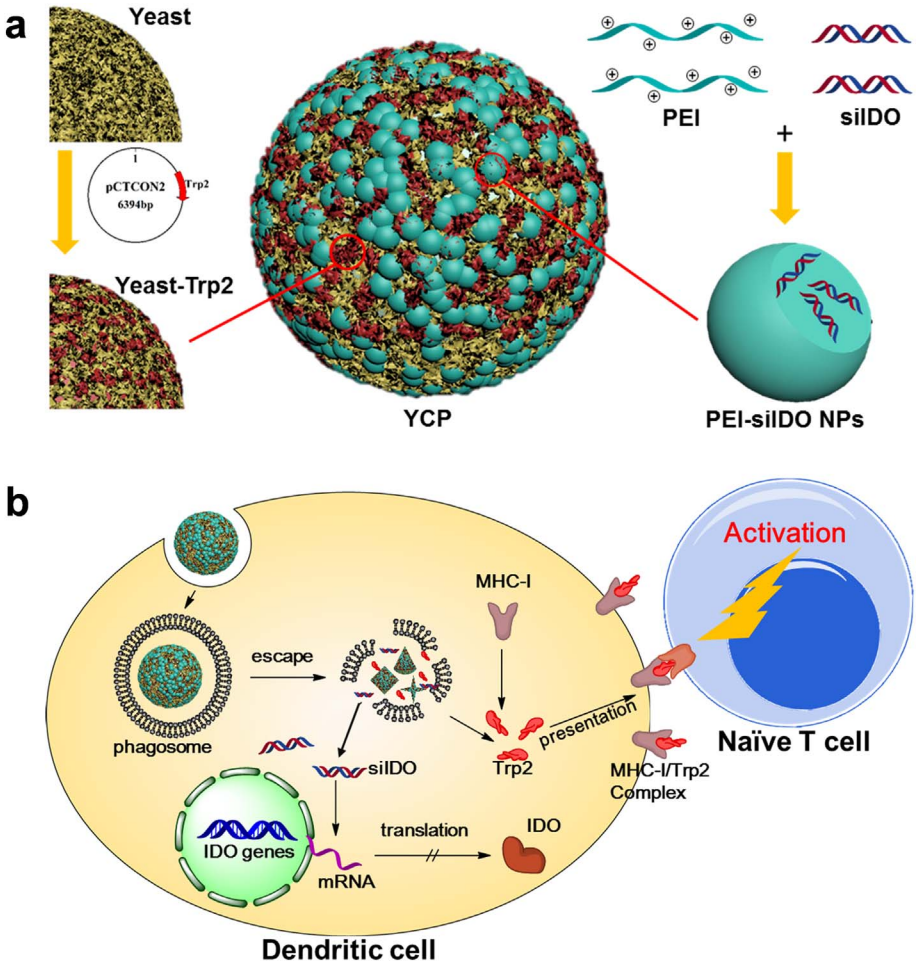

Fig. 1. Schematic illustration of the siRNA nanoparticle-coated *Saccharomyces cerevisiae* yeast-Trp2 and immune activation. (a) Schematic illustration of the construction of YCP. (b) Schematic illustration of intracellular tracking of YCP.