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Plant viral nanoparticles-based HER2 vaccine: Immune response influenced by differential transport, localization and cellular interactions of particulate carriers

Sourabh Shukla [a, f, *], Jay T. Myers [e], Sarah E. Woods [a], Xingjian Gong [a], Anna E. Czapar [a], Ulrich Commandeur [i], Alex Y. Huang [e, f], Alan D. Levine [g], Nicole F. Steinmetz [a, b, c, d, f, h, **]

[a] Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA
[b] Department of Radiology, Case Western Reserve University, Cleveland, OH 44106, USA
[c] Department of Materials Science and Engineering, Case Western Reserve University, Cleveland, OH 44106, USA
[d] Department of Macromolecular Science and Engineering, Case Western Reserve University, Cleveland, OH 44106, USA
[e] Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106, USA
[f] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA
[g] Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106, USA
[h] Division of General Medical Sciences-Oncology, Case Western Reserve University, Cleveland, OH 44106, USA
[i] Department of Molecular Biotechnology, RWTH-Aachen University, 52064 Aachen, Germany

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ABSTRACT

Cancer vaccines are designed to elicit an endogenous adaptive immune response that can successfully recognize and eliminate residual or recurring tumors. Such approaches can potentially overcome shortcomings of passive immunotherapies by generating long-lived therapeutic effects and immune memory while limiting systemic toxicities. A critical determinant of vaccine efficacy is efficient transport and delivery of tumor-associated antigens to professional antigen presenting cells (APCs). Plant viral nanoparticles (VNPs) with natural tropism for APCs and a high payload carrying capacity may be particularly effective vaccine carriers. The applicability of VNP platform technologies is governed by stringent structure-function relationships. We compare two distinct VNP platforms: icosahedral cowpea mosaic virus (CPMV) and filamentous potato virus X (PVX). Specifically, we evaluate *in vivo* capabilities of engineered VNPs delivering human epidermal growth factor receptor 2 (HER2) epitopes for therapy and prophylaxis of $HER2^{+}$ malignancies. Our results corroborate the structure-function relationship where icosahedral CPMV particles showed significantly enhanced lymph node transport and retention, and greater uptake by/activation of APCs compared to filamentous PVX particles. These enhanced immune cell interactions and transport properties resulted in elevated HER2-specific antibody titers raised by CPMV- vs. PVX-based peptide vaccine. The 'synthetic virology' field is rapidly expanding with numerous platforms undergoing development and preclinical testing; our studies highlight the need for systematic studies to define rules guiding the design and rational choice of platform, in the context of peptide-vaccine display technologies.

1. Introduction

Cancer vaccines are designed to activate or rejuvenate the immune system to recognize tumor-associated antigens and eliminate residual or recurring disease following primary treatments [1–3]. In stimulating a sustained endogenous immune response and resultant memory, cancer vaccines have the potential to overcome the limited, short-term effects associated with passive immunotherapies and the accompanying need for frequent administration

* Corresponding author. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA.
** Corresponding author. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA.
E-mail addresses: sourabh.shukla@case.edu (S. Shukla), nicole.steinmetz@case.edu (N.F. Steinmetz).

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at high cost. Passive immunotherapy, often administered systemically, is also associated with development of resistance and toxicities [4,5].

Several cancer vaccines have already been successfully incorporated in the clinic, and many different approaches are currently under development [3,6]. Peptide subunit-based vaccines are among the most explored cancer vaccine approaches and rely on the efficient presentation of epitopes to the various components of the immune system, a critical role of vaccine delivery platforms [7–11]. Nanoparticulate carriers are particularly promising candidates capable of delivering high payloads of peptide antigens with enhanced stability and bioavailability [12–14]. Moreover, particulate carriers can provide additional immunostimulatory impetus by engaging pattern recognition receptors on immune cells, thereby enhancing the overall immunogenicity of the vaccine [9,15].

Plant viral nanoparticles (VNPs) possessing highly ordered and multivalent protein capsids are ideally suited to display repetitive arrays of immunogenic peptide epitopes as vaccine platforms [16–21]. Conceptually different from viral vector platforms that rely on expression of antigenic peptides by antigen presenting cells (APCs) [22–25], VNPs can deliver large payloads of genetically fused or chemically conjugated immunogenic epitopes to a wide range of APCs [18,26]. In addition, the physical and genetic stability of VNPs and their non-integrating and non-infectious nature in mammals adds a layer of safety for VNP-based vaccine applications. VNPs can also be engineered to co-deliver other immunostimulatory molecules to improve vaccine efficacy [9,27].

A unique advantage offered by VNPs as vaccine platforms is their intrinsic immune-stimulatory properties that obviate the need for toxic adjuvants and co-stimulatory molecules [28]. However, the extent and nature of VNP-immune cell interactions has been shown to be dependent on particle morphology and molecular composition. The wide array of different shapes, sizes and aspect ratios (ratio of length and width) of VNPs bring about significant changes in *in vivo* properties and functionality. This strong structure-function relationship determines the suitability of one VNP over another for specific biomedical applications [19,20,29,30]. High aspect ratio nanoparticles offer significantly higher payload carrying capacity, but may also evade phagocytic immune cells (thus providing advantageous properties for drug delivery and imaging applications) [18,29–31]. Low aspect ratio materials, such as icosahedral platforms, may be beneficial for application as vaccines and immunotherapies [32].

In this study, we set out to evaluate VNP–immune cell interactions, define their fates *in vivo*, and evaluate their potential to trigger a human epidermal growth factor receptor 2 (HER2)-targeted humoral response. We compared two morphologically distinct VNP platforms: the 30 nm icosahedral cowpea mosaic virus (CPMV) and 515 × 13 nm filamentous potato virus X (PVX). Each particle platform was produced through farming in plants and chemically modified to display HER2-specific antigens. The immunological properties of the vaccine formulations were evaluated in tissue culture and in murine models.

We chose CPMV and PVX, because both platforms have been previously studied as vaccine delivery platforms in conjugation with epitopes derived from tumor antigens or infectious agents, demonstrating efficacy both to prime humoral and cellular responses in the context of cancer [32–38]. For example, PVX coupled with weak idotypic tumor antigen has been shown to induce protective humoral immunity against murine B-cell malignancy [38]. Furthermore, both platforms have been shown to show efficacy when applied as in situ vaccine for treatment of cancer: CPMV stimulates a potent systemic anti-tumor immune response in mouse models of melanoma, ovarian, colon and breast cancer [28]; and we recently demonstrated that PVX also elicits anti-tumor immunity when administered intramurally in a dermal melanoma model [Lee, Murray et al., in review].

We chose to target HER2 positive disease, because HER2 overexpression is associated with aggressive breast cancer (and other malignancies). Patients with this disease have a high incidence of metastasis development and relapse [39]. Successful implementation of passive immunotherapy with the HER2-specific monoclonal antibody Herceptin is a testimonial to the potential of antibody-mediated therapeutic intervention [40], and several other B cell epitopes from the extracellular domain of HER2 have been identified and are undergoing testing for vaccine development [41–44]. With the long-term goal to establish a VNP-based HER2 vaccine for treatment of $HER2^+$ patients, either used as a therapeutic or prophylactic vaccine, we initiated this project to assess the suitability of the platform technology, CPMV vs. PVX, for such development.

2. Results and discussion

2.1. Propagation and purification of CPMV and PVX particles

CPMV and PVX particles were propagated and purified using established methods [45]. The isolation of either VNP yielded approximately 1 mg of virus particle per gram of infected leaf material. TEM images show the distinct morphology of the two particles (Fig. 1A). CPMV is a 30 nm-sized nanoparticle (Fig. 1A) containing 60 copies each of a large (L, 42 kDa) and small (S, 24 kDa) coat protein arranged with pT = 3 icosahedral symmetry. PVX is a flexible filament measuring 515 × 13 nm (Fig. 1A) and is composed of 1270 identical copies of a 25-kDa capsid protein. Both CPMV and PVX particles can be stably stored for long periods of time (months-to-years). The physical and genetic stability as well as batch-to-batch structural consistency confer advantageous characteristics for application as a nanocarrier. For cellular uptake studies, fluorescently tagged CPMV and PVX particles were synthesized using established protocols targeting solvent-exposed lysine side chains using NHS-active Alexa Fluor 647 dyes (yielding A647-CPMV and A647-PVX). UV–vis spectroscopy was used to determine the degree of labelling: A647-CPMV was found to display 27 A647 per particle, and A647-PVX was found to display 175 A647 per particle. The 6-fold difference in labelling reflects the 6-fold greater molecular weight of PVX. Thus, the spatial array of fluorophores is displayed at a similar density yielding particles with comparable fluorescent properties.

2.2. Determination of VNP–APCs interactions

Interaction with and activation of professional APCs including dendritic cells (DCs) and macrophages are crucial for vaccine efficacy [46–48]. DCs sample antigens, transport immunogenic components (including vaccine carriers) to secondary lymphoid organs, initiate and sustain humoral and/or cellular responses. Thus efficient delivery of antigens to DCs is a critical step in vaccine-mediated immune stimulation. To compare the extent of endocytic uptake by DCs, fluorescently tagged CPMV and PVX particles (A647-CPMV and A647-PVX) were incubated with bone marrow derived DCs (BMDCs) isolated from FVB mice and analyzed using flow cytometry. Percentage of $CD11c^+$VNP + cells was then quantified as a measure of DC uptake (Fig. 1B). The results indicated a significantly ($p < 0.01$) higher percentage of $CD11c^+$ DCs were CPMV+ (36%) vs. PVX+ (12%), suggesting enhanced uptake of CPMV particles by DCs (Fig. 1B). The differential uptake was also evident through confocal microscopy, where BMDCs stained with anti-CD11c antibodies showed increased uptake of A647-CPMV particles over A647-PVX particles (Fig. 1C).

Uptake of antigens and particulate carriers by APCs depends on