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Protein-Based Nanoparticles in Cancer Vaccine Development

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Abstract

Peptide and protein-based cancer vaccines usually fail to elicit efficient immune responses against tumors. However, delivery of these peptides and proteins as components within caged protein nanoparticles has shown promising improvements in vaccine efficacy. Advantages of protein nanoparticles over other vaccine platforms include their highly organized structures and symmetry, biodegradability, ability to specifically functionalize at three different interfaces (inside, outside, and between subunits in macromolecular assembly), and ideal size for vaccine delivery. In this review, we discuss different classes of virus-like particles and caged protein nanoparticles that have been used as vehicles to deliver and increase the interaction of cancer vaccine components with the immune system. We review the effectiveness of these protein nanoparticles towards inducing and elevating specific immune responses, which are needed to overcome the low immunogenicity of the tumor microenvironment.

Text for Graphical Abstract: In this review, we discuss several different protein-based nanoparticles as delivery vehicles to increase the interaction of cancer vaccine components (e.g., adjuvants, tumor-associated antigens) with the immune system. These important components can be efficiently internalized and processed by dendritic cells, which then present the antigen to the T cells for specific T cell responses that lead to specific tumor lysis and elimination. The elevated immune responses that are elicited by these nanoparticle vaccines are advantageous to overcome the low immunogenicity of the tumor microenvironment.

Keywords

cancer vaccines; virus-like particles; caged protein nanoparticles; tumor antigens

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Introduction

Boosting a patient's native immune system by immunotherapy has been a promising approach in cancer treatment.[1] The goal of cancer vaccines is to promote the immune system to recognize distinct antigenic markers expressed primarily by cancer cells and to target such cells for lysis.[2] These markers, known as tumor associated antigens (TAAs), vary widely among different cancer types, and the identities of many TAAs have been elucidated.[3] In many clinically-examined cancer vaccines, TAAs are co-administered with adjuvant, which are immune activator molecules. Although these cancer vaccines have been shown to elicit an immune response, the clinical outcome is usually weak and insufficient to overcome the low immunogenicity of the tumor microenvironment.[1]

In recent years, different strategies have been developed to increase vaccine efficacy, such as vaccination with the whole tumor lysate,[4] combination of antigens with adjuvants,[5] and formulation in carriers such as nanoparticles (e.g., PLG, PLGA, gold nanoparticles),[6–8] liposomes,[9] and microparticles.[10] Virus-like particles (VLP) and caged protein (CP) nanoparticles have also attracted significant interest as cancer vaccine platforms for inducing antigen-specific immune responses against cancerous cells. We define VLPs as protein structures isolated from viruses which are lacking the infectious viral genome to a mammalian host, and CPs as self-assembled protein structures with physical properties and geometries similar to viruses but not from a viral source. VLP and CP nanoparticles as vaccine platforms have the potential to improve vaccine efficacy by promoting antigen localization to dendritic cell-enriched draining lymph nodes,[11] enhancing endocytosis of antigens by antigen presenting cells (APCs),[12] and increasing antigen presentation to the adaptive immune cells.[13] In this review, we discuss the different types of VLP and CP nanoparticles and their physical properties, biodistribution, and cellular uptake towards enhancing vaccine efficacy. Although VLP and CP nanoparticles have also shown success as platforms to induce higher immune responses to infectious diseases, vaccines for communicable diseases have been reviewed by others,[14,15] and will not be covered in this discussion.

Antigen-based Cancer Vaccines

Tumor-associated antigens (TAAs) are antigenic proteins produced by tumor cells which can trigger an immune response in the host.[16] Immunotherapy using vaccines is based on the premise that TAAs can induce specific cytotoxic T cell responses to cancer cells, resulting in tumor destruction without harming normal cells.[17] However, clinically-examined cancer vaccines, consisting of whole tumor antigen (proteins) or epitopes (smaller peptides), are often insufficient to overcome the low immunogenicity of the tumor microenvironment.[16]

To address this limitation, different approaches have been examined to increase the antitumor responses for improved vaccine efficacy. One strategy is the combination of these antigen-based cancer vaccines with immune activator molecules known as adjuvants.[17,18] Common adjuvants used in clinical trials include aluminum salts, oil-in- water emulsions (MF59), and monophosphoryl lipid A (MPL) with aluminum salt.[19] Recently, ligands of Toll-like receptors in APCs such as CpG[20–22], poly-IC[23,24], and imidazoquinoline[25,26] have

also attracted considerable interest as cancer vaccine adjuvants in preclinical and clinical trials.

Alternative approaches for increasing vaccine efficacy such as using multiple antigen peptide epitopes[27–29] and personalized peptide formulations have been developed and supported by clinical studies.[30,31] Vaccination with multiple-peptide epitopes from different sources can decrease the possibility of tumor escape and increase the anti-tumor responses relative to single epitope immunization.[32,33] Also, since tumor cells and TAAs are heterogeneous among patients, a personalized selection of peptides against individually-expressed antigens can increase efficacy.[34]

Despite these improvements, clinical outcomes of cancer vaccines have still been limited by factors such as identification of optimal antigens, adjuvants, and importantly, delivery system. Our focus in this review is to discuss and survey VLP and CP nanoparticles that have been used as delivery systems to increase effectiveness of cancer vaccines.

Delivery Systems

Generation of potent specific immune responses to cancer is dependent on antigen uptake by APCs, particularly dendritic cells (DCs). Efficient uptake by DCs is subject to the important antigen properties of size, shape, and surface charge.[35,36] Additional key steps in generation of response include proper activation of DCs, trafficking of DCs to lymph nodes (LNs), sufficient communication of DCs with adaptive immune cells such as CD8 T cells, and activation of cytotoxic T cells for targeted tumor lysis (Figure 1).[37]

The use of nanoparticles in vaccines is supported by the premise that a higher cellular uptake and an elevated interaction of antigens with the immune cells can be achieved by using an optimally-designed delivery system.[38] Vaccine delivery materials that have been examined for cancer immunotherapy include liposomes, polymers, nanoparticles, and hydrogels.[8,39] Nanoparticles based on proteins, in particular VLPs and CPs, have symmetries and physical properties that are similar to viruses and can potentially increase the interaction of the vaccine components with APCs. We briefly discuss how delivery system properties, such as size, shape, and surface charge, can affect the cellular uptake and induce potentially more effective anti-tumor immune responses.

Size—Nanoparticle studies have shown that there is an optimal size range for passive transport to the lymphatic system and APCs.[36] Particles between 20–45 nm are drained significantly by LNs, with a relatively high retention time measured up to 120 hrs post-injection.[40] Particles of this size range are internalized by almost 50% of LN-resident DCs compared to 10% internalization by APCs of 100-nm particles.[40–42] However, particles below 10 nm are not internalized by DCs efficiently.[36] Furthermore, relatively high (~76%) lymphatic uptake was observed for 40-nm liposomes compared to larger liposomes (>150 nm), the latter of which remained almost completely at the site of injection.[43]

As discussed, conventional formulations of peptide- and protein-based cancer vaccines usually yield relatively weak immune responses, which can be attributed to insufficient uptake and interaction of antigens with APCs.[36] The size-uptake studies suggest that

delivering the soluble protein or peptide antigens (which are typically much smaller than 5 nm) within nanoparticles of ~20–50 nm can lead to more efficient lymphatic drainage and a higher antigen uptake by DCs, resulting in stronger adaptive immunity to the antigens.

Shape—Particle shape can also affect the uptake of nanoparticles by immune cells.[36,44,45] Although investigations have shown that non-spherical particles such as rod-shaped particles have higher circulation times, they also demonstrated decreased cellular uptake compared to spherical NPs.[46] In fact, spherical NPs have the highest cell internalization rate compared to cubic, rod and disk-like shaped NPs.[47] Spherical polystyrene particles (diameters ~200 nm) conjugated to ovalbumin antigen (OVA) generated stronger *in vivo* Th1 and Th2 immune responses relative to rod-shaped particles.[48] In addition, enhanced LN transport and uptake by APCs was observed for the spherical virus-like particle, cowpea mosaic virus (CPMV), compared to the rodshaped virus-like particles, potato virus X (PVX).[49] Others have demonstrated that not only shape but the initial orientation of particles can affect phagocytosis by macrophages.[45]

Surface charge—Surface charge is another parameter that can affect the cellular uptake of NPs.[36,50] Neutral NPs demonstrated minimal cellular interaction and cellular uptake compared to the charged NPs.[50] Positively charged nanoparticles have the greatest efficiency in cellular internalization, possibly due to their interactions with the negatively charged groups on the cell membrane.[50] However, evidence also demonstrated uptake of negatively charged nanoparticles despite their unfavorable electrostatic interaction with cell membranes. For example, cellular uptake of both negatively and positively charged micellar NPs has been observed through different endocytic pathways *(e.g.,* clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis).[51]

With regard to tumor tissue, *in vivo* biodistribution of chitosan and micellar-based NPs suggested that NPs with relatively weak negative surface charges tend to accumulate in tumors more than NPs with positive or highly-negative zeta potential values;[51,52] this is somewhat consistent with a recent extensive survey demonstrating that NPs with neutral surface charges tend to result in higher delivery efficiencies to tumors, relative to NPs with more positive or negative charges.[53] An important, but sometimes overlooked, consideration is the adsorption of blood components on nanoparticle surfaces (called the protein corona), which can modify NP surface properties. Therefore, physicochemical properties of a NP immediately after synthesis can be different than the one that cells encounter *in* vivo.[54–56]

Virus-Like Nanoparticles

Viruses activate immune responses due to their repetitive surface structure and presence of pathogen associated molecular patterns (PAMPs).[57] PAMPs are viral and microbial components that are recognized as foreign by the pattern recognition receptors, and their detection leads to a cascade of cytokine production and activation of innate immunity. VLPs share these similar components as viruses, providing an efficient platform to enhance immunogenicity and stability of low immunogenic antigens. Furthermore, they exhibit an additional advantage of lacking mammalian-replicable genetic material, rendering VLPs to be non-infectious to the host. VLPs have been explored as delivery systems for cancer

vaccines (see Table 1), and this section will specifically focus on icosahedral plant viruses, rod-shaped plant viruses, and bacteriophage Qβ (Figure 2A). Specific examples are also highlighted in Figures 3A and 3B.

Icosahedral plant viruses—One of the most studied icosahedral plant viruses is the cowpea mosaic virus (CPMV) which is a 28-nm capsid composed of 120 protein subunits (60 large and 60 small). CPMV has been shown to bind and be internalized by APCs *in vitro* and *in vivo*.[58] Intraperitoneal injection of CPMV resulted in localization of these virus nanoparticles in the lymph nodes and APCs, leading to APC activation.[49] Furthermore, in different metastatic cancer models, vaccination with empty CPMV without any antigens led to a longer survival time in mice. This unusual observation was correlated with an increase in the recruitment of tumor-infiltrating neutrophils and the production of cytokines that activate adaptive immune responses.[59] In these mice immunized with CPMV, no noticeable signs of injury or inflammation were observed in the histology of reported organs.[59]

Attachment of cancer antigens onto CPMV has been effective in inducing antigen-specific responses. Subcutaneous immunization with CPMV conjugated with HER2 breast cancer epitopes (CH401 and P4) significantly increased HER2-specific antibody responses and tumor protection in murine models (Figure 3A).[49] Delivery of low immunogenic Tn antigen, a tumor-associated carbohydrate antigen found in various cancers, with Freud's adjuvant enhanced the production of Tn-specific IgG antibodies; these antibodies were capable of recognizing breast cancer cells.[60]

Other icosahedral plant viruses, such as alfafa mosaic virus and cowpea chlorotic mottle virus (CCMV), are also being engineered for antigen delivery. Antigen- conjugated incorporation of the two plant viruses have shown to elicit specific antibody and $CD8^+$ T cell responses to infectious diseases.[61,62] Although applied to communicable diseases, the generation of high antigen specific $CD8^+$ T cell responses opens a promising potential of using those icosahedral plant viruses in cancer vaccines.[63]

Rod-shaped plant viruses—Some of the rod-shaped plant viruses investigated as vaccine constructs include tobacco mosaic virus (TMV) and potato virus X (PVX). These viruses have dimensions of 300 × 18 nm and 515 × 13 nm, respectively, and the filamentous structure and high aspect ratio of TMV has been reported to enhance tumor homing and penetration for drug delivery and tumor imaging.[64] Furthermore, the large surface areas (relative to icosahedral viruses) allow for presenting a greater number of antigens and conjugating larger antigens. TMV and PVX showed efficient DC uptake which resulted in DC activation,[65,66] and immunization with epitope-conjugated TMV in murine models increased the specific CD8 T cell responses in different infectious diseases.[15]

Vaccines using tumor-associated epitopes have also been developed with TMV and PVX. Immunization of Tn-conjugated TMV at tyrosine 139 resulted in higher Tn- specific IgG and IgM antibody responses when co-administered with Freund's adjuvant.[67] However, attachment of target antigens to the N-terminus of TMV monomers did not yield in any immune response,[67] suggesting the importance of conjugation site in inducing immune responses. It was also reported that bivalent conjugation of two melanoma antigens (p15e

and Trp2) to TMV increased tumor protection, compared to a mixture of monovalently conjugated p15e-TMV and Trp2-TMV. Antigen delivery (SIINFEKL or p15e) with TMV through reducible chemical conjugation and genetic modification was compared, and it was observed that the genetic insertion of antigen to TMV was not as effective in eliciting an immune response as chemical conjugation.[69]

PVX has also been used as an antigen delivery platform for different models of cancers. The entire idiotypic (Id) tumor antigen, derived from BCL1 lymphoma, has been presented on PVX through the streptavidin-biotin interaction. Id-tumor antigens are immunogenically weak, but immunization with Id-PVX resulted in higher anti-Id IgG antibody responses and higher survival rate after lymphoma challenge.[70] HER2 breast cancer epitopes (CH401 and P4) have also been conjugated with PVX to generate increased antibody responses in murine model compared to soluble free epitopes.[49]

Bacteriophage Qβ—Bacteriophage Qβ is an *E. coli* RNA phage with a 25-nm self-assembled icosahedral capsid that consists of 178 capsid proteins. Vaccination with epitope-conjugated Qβ has led to activation of DCs, increase in pro-inflammatory cytokines, and antibody responses when formulated with CpG adjuvant.[71] The toxicology of Qβ and the use of therapeutic Qβ for chronic disease immunotherapy have been reviewed elsewhere.[72]

Qβ has shown promise in antigen-specific cancer immunotherapy for several cancer models. Tumor-associated carbohydrate antigens have been conjugated to Qβ and resulted in increased survival of mice challenged with mammary tumor cells (Figure 3B).[73] Melanoma-specific Melan-A/Mart-1 peptide was conjugated to Qβ with TLR9 adjuvant CpG (particle formulation CMP-001), and its efficacy has been examined in HLA-A2 transgenic murine models as well as in melanoma patients in a phase I/II clinical investigation.[74] A phase II clinical study was performed using the same melanoma antigens with IFA and imiquimod, and 16/21 patients generated specific T cell responses *ex vivo*,[75] with only mild or moderate local injection site responses reported.[75] The same vaccine formulation is currently in two clinical studies examining combination therapy with blockade antibody anti-PD1 (Pembrolizumab) in patients with advanced stage melanoma (NCT03084640[76] and NCT02680184[77]).

Caged Protein Nanoparticles

As previously described, highly-organized structures and symmetries of caged protein (CP) nanoparticles, their biodegradability, and their optimal size for delivery make them attractive vaccine platforms. CP NPs are protein assemblies that have virus-like structures and geometries, but are not from viral sources. Examples of CP NPs that have been used in the field of cancer immunotherapy are heat-shock proteins, E2, ferritin, and protein vault nanoparticles (Figure 2B), and we discuss these below. A summary is also presented in Table 1, with examples in Figures 3C and 3D.

Heat-shock proteins—Heat-shock proteins (HSPs), the most abundant class of chaperone proteins, are produced by cells in response to stress conditions.[78] HSPs range in molecular sizes from 8 to 150 kDa, and are classified based on their molecular weights *(e.g.,* hsp40, hsp70, hsp110).[79] These proteins have been used as platforms for drug delivery.[80]

Furthermore, HSPs are overexpressed in a wide range of human cancers, and they are often involved in tumor cell proliferation, invasion, and progression.[81]

This overexpression of HSPs on tumors has made them an attractive source for anticancer vaccines. It has been observed that HSPs isolated from tumor cell lysates are bound to cancer antigens from the parental tumor.[82] Therefore, one approach in using HSPs for cancer treatment is to extract these HSP-tumor antigen complexes from tumors and immunize with them.[83] In this strategy, the tumor antigens bound to HSPs will be taken up more efficiently by DCs[84] and activate the typical process described in Figure 1.[84]

Vaccination with HSP-tumor antigen complexes has been investigated for different cancer types in preclinical and clinical studies, and has primarily resulted in an increase in the CD8 T cell responses.[85–89] There are also still ongoing clinical trials using autologous HSP-tumor antigen complexes, including gastric (NCT02317471),[90] glioblastoma (NCT02122822),[91] and liver (NCT02133079)[92] cancers. Although studies have demonstrated efficacy and safety, one major limitation is the overall yield; the amount of vaccine obtained is dependent on the volume of tumor tissue isolated from the patients.[83]

The use of recombinant protein in designing HSP cancer vaccines partially addresses this yield limitation of tumor-derived HSPs. Recombinant HSPs serve as a carrier for the antigens of interest, which can be loaded through the chaperone-binding properties.[93] Immunization of mice with recombinant HSPs containing gp100,[93] MAGE, [94] or HER2[95] antigens resulted in a higher level of antigen-specific IFN-γ production, supporting T-cell activity. This translated to an increase in survival time for tumor- bearing animals immunized with HSPs-antigen complexes.[93,94]

E2 Protein Nanoparticle—The self-assembled protein nanoparticle E2 is derived from the E2 subunit of the pyruvate dehydrogenase complex from *Bacillus stearothermophilus.* It has been used in drug delivery and vaccines.[96–99] The assembled NP is composed of 60 identical monomers that form a highly thermostable dodecahedral caged structure[100] with a diameter of 25 nm, which is within the favored size range for lymphatic transport and DC uptake.[40,41] This caged structure has an internal 12-nm cavity and twelve 5-nm openings leading to this hollow cavity. The scaffold has three interfaces (internal hollow cavity, subunit-subunit interface, and the exterior surface) which can be molecularly modified for site-directed functionalization.[99–101]

Biodistribution studies have shown that E2 is taken up effectively by DCs, with almost 50% of DCs within the draining LNs being associated with E2 NPs at 6 hrs post-injection.[11] Furthermore, an even higher *in vitro* and *in vivo* uptake by APCs was observed when E2 was designed with DNA attached to the surface, compared to E2 alone.[11] This efficient uptake by DCs suggests that the E2 NP could be an effective platform for the development of cancer vaccines.

Supporting this premise, studies have demonstrated a significantly higher DC activation and antigen cross-presentation when an OVA antigen (SIINFEKL) and DC- activating DNA (CpG) are both attached to E2 (Figure 3C, upper left).[13] Simultaneous temporal and spatial

delivery of OVA and CpG to DCs increased CD8 T cell activation *in vitro*,[13] with concurrent delivery of antigen and CpG being essential to achieve the highest T cell activation.[13] *In vivo* studies in C57BL/6 mice showed higher antigen- specific CD8 T cell proliferation and IFN-γ secretion when an epitope of the TAA gp100 was co-delivered with CpG using E2, compared to free antigen and CpG.[99] This enhanced activity translated to an increased animal survival time in the aggressive B16- F10 melanoma tumor model.[99]

Applicability for solely-human TAAs was also reported. In a transgenic mouse model humanized with the HLA-A2 gene, significantly higher IFN-γ secretion and cell lysis activity were observed when the immunodominant epitopes of HLA-A2 restricted human cancer-testis antigens and CpG were coupled to E2 (Figure 3C, upper right and bottom).[102] Furthermore, combined delivery of cancer epitopes from different antigen sources within E2 yielded an additive effect that increased lytic activity towards human cancer cells bearing the antigens.[102] These investigations demonstrate that formulation of TAAs within E2 NPs can significantly enhance cell-mediated immune responses.

Ferritin—Ferritin protein cage nanoparticles self-assemble from identical subunits; for example, ferritin isolated from *Pyrococcus furiosus* comprises 24 subunits, forming a 12-nm diameter protein complex with a hollow cavity of 8 nm.[103] Ferritins have been used for drug delivery, imaging, and targeting applications.[104,105] A recent study demonstrated that ferritin protein cages carrying ovalbumin peptides were efficiently phagocytosed by DCs and resulted in a high specific CD8 T cell induction which selectively killed antigen-specific target cells.[103] Human ferritin can also deliver red fluorescence protein (RFP) efficiently to LNs, with a high retention time up to six days after injection.[106] This passive targeting to LNs resulted in higher RFP-specific cytotoxic CD8 T cell responses, which decelerated growth of RFP-expressing melanoma cells *in vivo* and increased animal survival time (Figure 3D).[106]

Protein vault nanoparticles—Vault nanoparticles are mammalian self-assembling ellipsoidal structures with an internal hollow cavity. These particles are highly uniform and are approximately 40 nm in width and 70 nm in length, with a mass of ~13 MDa.[107–109] Vault nanoparticles have been used in applications such as cell targeting[110] and drug delivery.[111] Recent investigations suggested that vault nanoparticles have adjuvant properties that favor cell-mediated over humoral-mediated immune responses, and therefore can be advantageous for use in cancer vaccines to induce cell-mediated responses.[112] A greater number of OVA $CD8^+$ memory T cells and a higher level of specific IFN-γ production was observed when the same dose of OVA antigen was delivered through vault nanoparticles compared to liposomes.[112] Elevated induction of CD8 T cells by vault nanoparticles could be a result of highly efficient internalization of these nanoparticles by DCs.[113]

Vault nanoparticles were also used to efficiently deliver CCL21 to the tumor microenvironment. This ligand plays an important role in the homing and localization of immune cells. Intratumoral injection of CCL21-modified DCs (with no vault nanoparticles) enhanced immune cell recruitment and inhibited lung tumor growth in a preclinical study.[114] Although this CCL21-modified DC treatment was somewhat effective, the extensive work to isolate and culture autologous DC, often with a low DC yield, is a limitation. As an

alternative strategy, vault nanoparticles were demonstrated to deliver the CCL21 ligand efficiently; this approach promoted the recruitment of T lymphocytes and DCs into the tumor microenvironment and resulted in antitumor activity towards an *in vivo* 3LL lung cancer model.[114]

Possible Immune Responses to the Delivery Platforms

Although immunogenicity is important in vaccine delivery, immune recognition to the nanoparticle platform itself could be a potential problem that leads to an antibody response resulting in neutralization and rapid clearance of the vaccine.[116] Administration of protein nanoparticles such as CCMV, CPMV, and HSPs have yielded higher B cell counts and specific IgG antibody titers, which resulted in rapid clearance.[117,118]

Approaches to slow down clearance of different protein-based delivery systems have been investigated. PEGylation of particles such as PVX and bacteriophage Qβ led to increased plasma circulation and reduced non-specific immune recognition.[66,119] With recent studies suggesting anti-PEG antibody production after repeated administration of PEGylated nanoparticles,[120] alternatives to PEG are being developed. For example, the “self-marker” membrane protein CD47 ectodomain and its self-peptide have been displayed on nanobeads to avoid phagocyte-mediated clearance and resulted in 10-fold enhanced plasma circulation time.[121] Furthermore, CD47 peptide decorated on the surface of VLP was shown to decrease phagocytosis *in vitro*.[122] Other methods that mimic PEG using amino acids such as XTENylation[123] and PASylation[124] have also led to immune evasion and increased circulation time when conjugated to proteins, but further investigations are needed to validate these strategies on protein nanoparticles for antigen delivery.

Future Opportunities

Protein-based nanoparticle platforms present exciting opportunities to significantly improve cancer vaccines effectiveness. Co-delivery of high payloads of adjuvants and antigens within NPs promotes antigen-specific immune responses against cancers. While progress has been made in this field, there is still potential for improvement and for mechanistic understanding. One strategy to increase the efficacy of protein-based NP vaccines is combination of these vaccines with other FDA- approved treatments, such as blockade checkpoint inhibitors or immune-regulating drugs. An example of a drug with which to investigate co-delivery would be α-CTLA4 (ipilimumab); it was approved by the FDA in 2011 for treatment of patients with late-stage melanoma[125] and belongs to the class of checkpoint blockade antibodies (e.g., α- CTLA4, α-PD1) shown to enhance anti-tumor responses.[126,127]

Synergistic anti-tumor activity has been observed when checkpoint blockade treatments are combined with cancer vaccines that are formulated with the immune regulatory cytokine GM-CSF in PLG.[128] The synergistic effect results from the simultaneous boost in the T cell response (due to the vaccine), the recruitment of immune cells (e.g., APCs; due to GM-CSF), and a decrease in the T cell inhibition (due to the checkpoint inhibitor). Similar combination strategies of protein-based nanoparticle vaccines with other treatments also have strong potential to improve the efficacy of NP- based vaccines.[129]

Summary

The delivery of cancer antigens within protein-based NPs can potentially increase the antigen uptake and interaction by APCs, resulting in an increase in cell- mediated immune responses specific to the particular cancer antigen. These advantages are likely enabled by physical properties, co-delivery of bioactive chemical elements, and geometries of the NPs that are similar to viruses. Recent studies have shown that the effectiveness of antigen-based cancer vaccines is improved when NPs are used as delivery platforms, and we are now observing the emergence of this approach in immunotherapy-based vaccines. Future studies will reveal the feasibility of using protein-based NPs and their involvement in combination therapy in the clinical field of cancer treatment.

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Abbreviations:

APC	antigen presenting cell
CCMV	cowpea chlorotic mottle virus
CP	caged protein
CpG	unmethylated cytosine-phosphodiester-guanine rich single-stranded oligonucleotides
CPMV	cowpea mosaic virus
DC	dendritic cell
E2	caged E2-subunit assembly of pyruvate dehydrogenase
DC	dendritic cell
HSP	heat shock protein
Id	idiotypic antigen
LN	lymph node
MHC	major histocompatibility complex
NP	nanoparticle
OVA	ovalbumin
PVX	potato virus X

TAA	tumor associated antigen
RFP	red fluorescence protein
TMV	tobacco mosaic virus
VLP	virus-like particle
Qβ	bacteriophage Qβ

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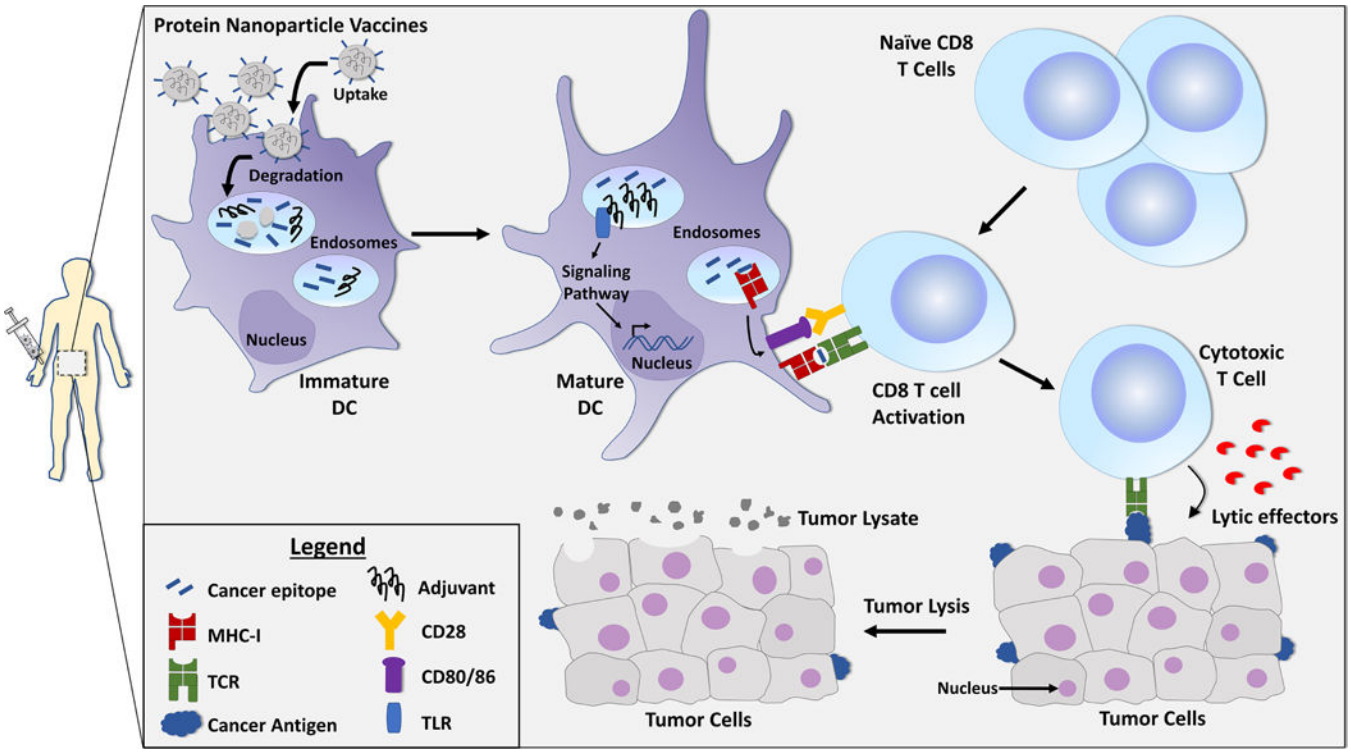

Figure 1. Common mechanism of tumor cell elimination.

Protein nanoparticle (NP) cancer vaccines that are injected *in vivo* can accumulate in the LNs and spleen. Immature DCs residing in these tissues internalize and degrade the NPs and process the antigens and adjuvants for potential danger signals. If DCs are activated through an adjuvant-TLR interaction, they present the antigens to the T cells in the context of MHC-I molecules for specific and longer-term T cell responses (i.e., cross-presentation). Upon T cell activation and recognition of tumor-associated antigens on cancer cells, T cells secrete lytic effectors (such as perforin), leading to tumor lysis and elimination. Abbreviations in the figure include: MHC-I (major histocompatibility complex, class I), TCR (T-cell receptor), CD28 (cluster of differentiation 28, costimulatory molecule), CD80/86 (cluster of differentiation 80/86, costimulatory molecules), TLR (Toll-like receptor).

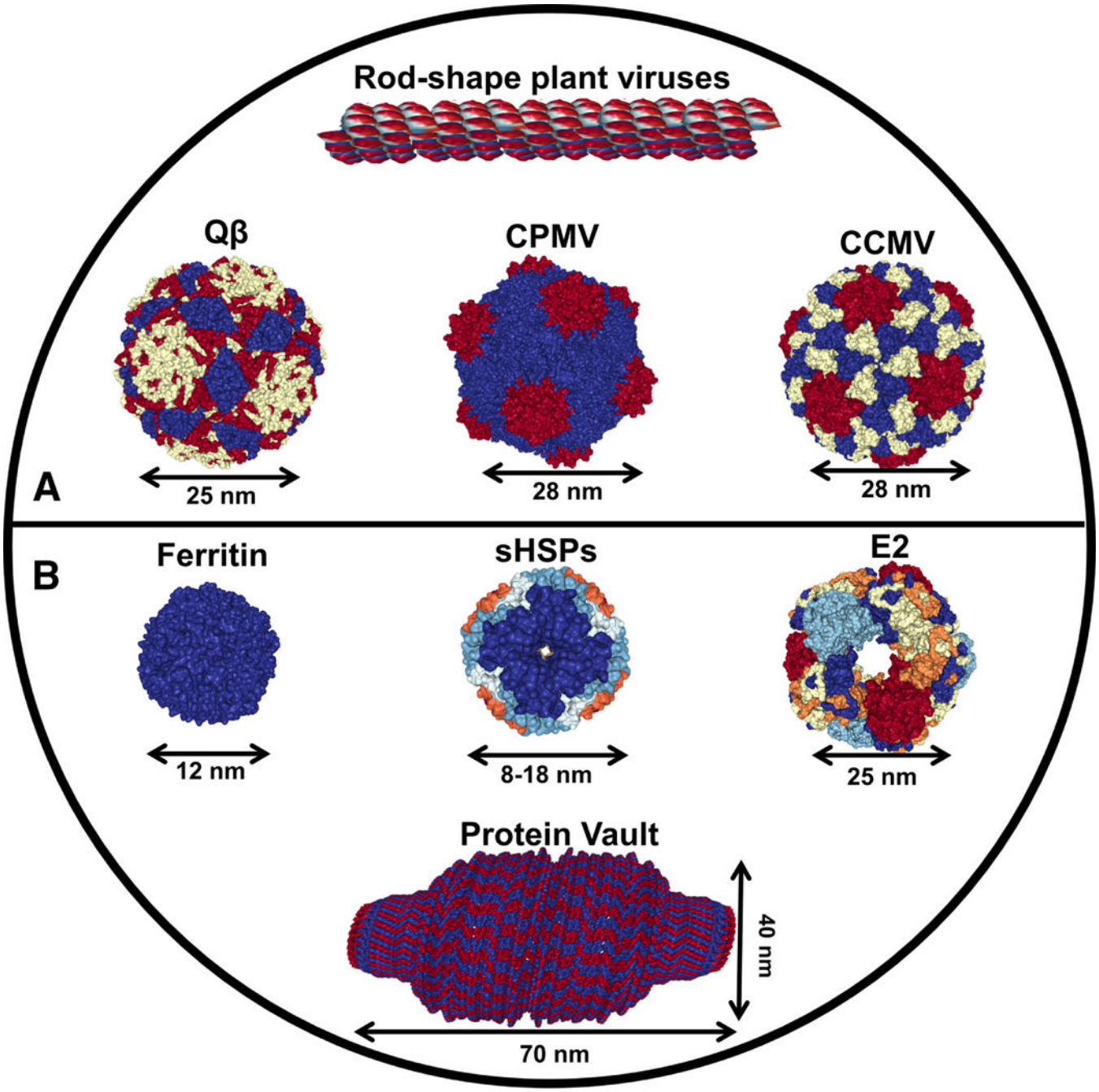

Figure 2. Protein structures of different virus-like particles (panel A), and caged protein nanoparticles (panel B).

Structural images are from Protein Data Bank (PDB; http://www.rcsb.org/pdb/home/home.do). Structure of TMV is reconstructed from helical structure (PDB ID code: 3J06), Qβ (1QBE), CPMV (1NY7), CCMV (1ZA7), ferritin (1MFR), small HSPs (3VQK), E2 (1B5S), and protein vault (2QZV).

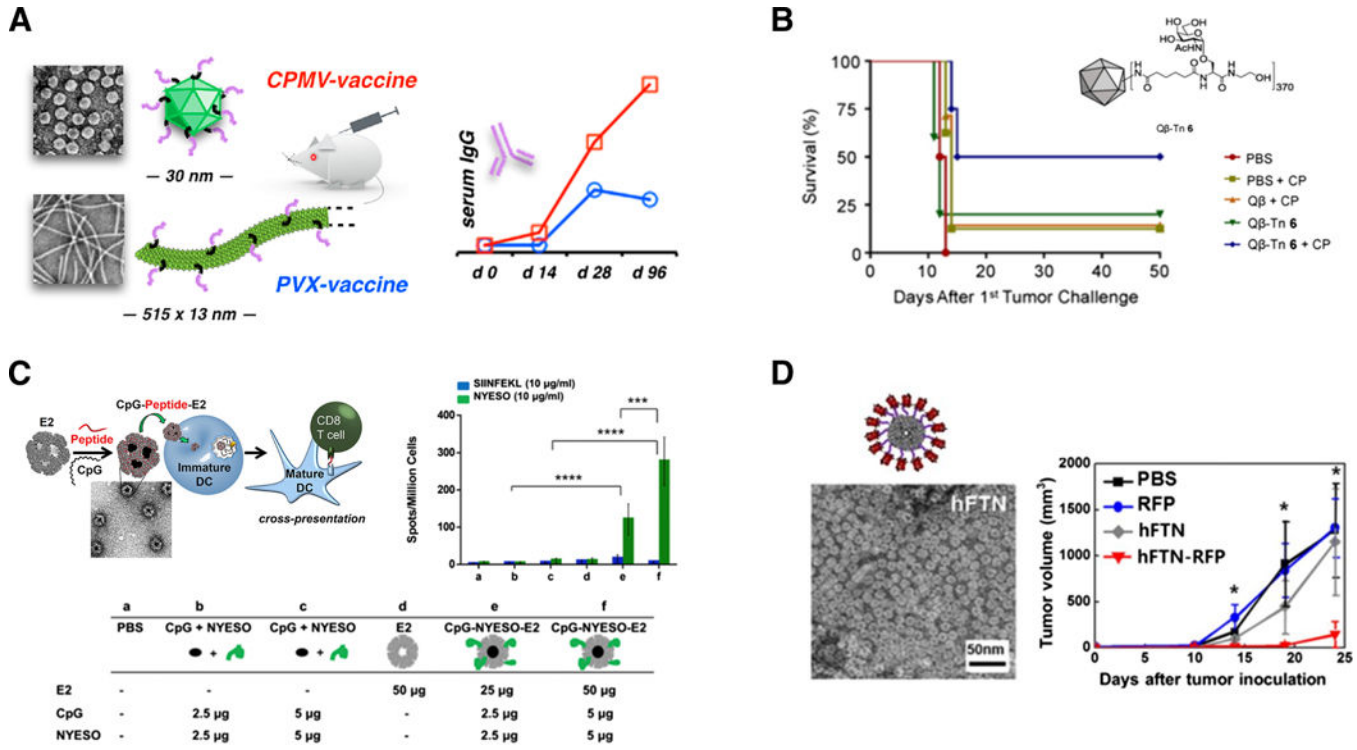

Figure 3. Examples of protein-based cancer vaccines.

(A) TEM images and antibody responses of plant virus-based cancer vaccines. HER2 antigen conjugated to CPMV and PVX nanoparticles resulted in higher antibody responses. Reprinted from Shukla et al., *Biomaterials* **121**, 15–27, copyright (2017); with permission from Elsevier. **(B)** Tumor-associated carbohydrate antigens conjugated to Qp resulted in increased survival of mice challenged with mammary tumor cells. Reprinted from Yin et al., *ACS ChemBiol.* **10**, 2364–2372, copyright (2015); with permission from American Chemical Society. **(C)** E2 nanoparticles in cancer vaccine studies. Upper left: Schematic of E2 interaction with immune cells. High DC activation and antigen crosspresentation result when antigen and DC-activating molecules are both attached to E2 nanoparticles. Reprinted from Molino et al. *ACS Nano* **7**, 9743–9752, copyright (2013); with permission from American Chemical Society. Upper right and bottom: Conjugation of human cancer-testis antigen and adjuvant to E2 nanoparticle increased specific IFN-γ secretion. Reprinted from Neek et al., *Biomaterials* **156**, 194–203, copyright (2018); with permission from Elsevier. **(D)** TEM image of ferritin nanoparticles (left). Immunization with red fluorescence protein (RFP) significantly decreased the RFP-expressing melanoma tumor growth in mice. Reprinted from Lee et al., *Scientific Reports* 6: 35182 (2016). doi:10.1038/srep35182. License for use at http://creativecommons.org/licenses/by/4.0/.

Table 1.

Summary of virus-like & caged protein nanoparticles that have been explored as delivery platforms for cancer vaccines.

Protein NP	Antigen	Adjuvant	Target/ Cancer	*In vivo/ In vitro*	Clinical Study	Response Investigated	Ref
CPMV	Her2	-	Breast	*In vivo*	-	Antibody	49
CPMV	Tn	Freund's	Breast, colon, prostate	*In vivo*	-	Antibody	60
PVX	Her2	-	Breast	*In vivo*	-	Antibody	115
PVX	Recombinant Id I	Alum	B-cell lymphoma	*In vivo*	-	T cell and antibody	70
TMV	Tn	Freund's	-	*In vivo*	-	Antibody	67
TMV	SIINFEKL	CpG	-	*In vitro, In vivo*	-	T cell	68
TMV	p15e	CpG	Melanoma	*In vitro, In vivo*	-	T cell	69
Bacteriophage Qβ	Melan-A26–35	CpG and IFA	Melanoma	*In vivo*	Yes	T cell	74, 75
Recombinant HSP 110	Her2/Neu	-	-	*Ex vivo*	-	T cell and antibody	95
Recombinant HSP 70	MAGE-A1	-	Melanoma	*Ex vivo, In vivo*	-	T cell	94
Recombinant HSP 110	gp100	-	Melanoma	*Ex vivo, In vivo*	-	T cell	93
Tumor derived HSP96	-	-	Colorectal		yes	T cell	85
Tumor derived HSP96	-	-	Melanoma		yes	T cells	86
Tumor derived HSP96	-	-	Glioblastoma		Yes	T cell	88
Tumor derived HSP70	-	-	Lung		Yes	NK cell	89
E2	SIINFEKL	CpG	-	*In vitro*	-	T cell	13
E2	gp100	CpG	Melanoma	*In vivo*	-	T cell	99
E2	NY-ESO-1	CpG	Melanoma	*Ex vivo*	-	T cell	102
E2	MAGE-A3	CpG	Melanoma	*Ex vivo*	-	T cell	102
Ferritin	SIINFEKL	-	-	*In vitro, Ex vivo*	-	T cell and antibody	103
Ferritin	RFP		RFP-Melanoma	*Ex vivo, In vivo*	-	T cell	106
Vault protein	SIINFEKL	-	-	*In vitro, In vivo*	-	T cell and antibody	112
Vault protein	CCL21 ligand	-	Lung	*In vivo*	-	Immune cells	114