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Therapeutic Delivery

Virus-like nanoparticles: emerging tools for targeted cancer diagnostics and therapeutics

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"The research and development of virus-like nanoparticles (VLNPs) exploits the structural characteristics of virus capsids as bionanotechnology platforms with great potential in drug delivery and molecular imaging."

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Viruses possess a rich array of functional nucleic acids and proteins. Genome size limitations and a lifestyle that requires surreptitious entry into cells, followed by rampant replication within them, have endowed viruses with a finely tuned capability to coordinate cellular components and processes. Our use of these features drives fundamental methodology in biotechnology. From exploiting the viral regulatory sequences that enable heterologous gene expression in all kingdoms of life, to the use of viral vectors to protect and deliver replicating nucleic acid therapeutics, virus-derived technologies have become commonplace. With increasing structural knowledge of different viruses comes an opportunity to co-opt nature's nanoscale delivery vehicle for small molecule and protein therapeutics. The research and development of virus-like nanoparticles (VLNPs) exploits the structural characteristics of virus capsids as bionanotechnology platforms with great potential in drug delivery and molecular imaging.

Virus coat proteins have evolved to mediate cell uptake of the bioactive components they contain and, in many cases, to escape from the endocytic pathway, releasing their cargo in the cytoplasm or nucleus. The primary infectious component of viruses, their genome, has traditionally been the main target for therapeutic delivery. Gene therapy vectors [1] and oncolytic viruses [2] have been widely explored in cancer treatment, relying on replicating genetic cargo to override cellular processes. In recent years, these biotechnologies have shown enormous potential, but also suffered significant setbacks. Unchecked replication and the potential for chromosomal integration of DNA vectors have required extensive engineering and careful preclinical assessment. Notwithstanding these caveats, the application of technologies based on replicating viruses has driven the development of capsid engineering to improve tissue or tumor selectivity; an approach that now feeds into the development of VLNPs as targeted delivery vehicles [3].

Virus-like nanoparticles

The term 'VLNPs' refers to the noninfectious protein shells, or capsids, comprised of virus-derived structural proteins, modified to be of use in nanotechnology. Depending on the cognate virus, VLNPs can be generated from productive infections of host material or by recombinant protein expression and self-assembly. Either approach can result in high yields and high purity of nanoparticles derived from a range of viruses that naturally infect bacteria, plants or animals. In the case of recombinant VLNPs, that is, those that are self-assembled from heterologous expression of structural proteins, the empty interior cavity is amenable to both genetic and chemical modification. Some VLNPs derived from productive infections can be disassembled and reassembled *in vitro*, allowing isolation from the infectious nucleic acid component. The distinguishing features of these biocompatible and biodegradable nanoparticles are symmetry, polyvalency and unrivaled monodispersity.

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Structural biology has long been associated with the biomolecular engineering of virus capsids and is a fundamental aspect of physical virology and the development VLNP-based technologies. A key advantage of VLNPs as nanoparticles is the availability of structural models with near-atomic detail; they enable the rational modification of capsid proteins with molecular precision by standard protein engineering techniques and bioconjugation chemistries, or a combination of these via the introduction of unnatural amino acid residues [4]. The deceptively simple architecture of virus capsids results in a powerful platform to re-engineer form and function, for example, tropism and cellular uptake pathway, or programmable cargo loading.

Cancer cell targeting

The bottom-up assembly of a large number of relatively few individual subunits results in precise repetitive presentation of functional protein structures that interact with ligands and host cells. In some cases, such as the specific interaction of the plant virus Cowpea mosaic virus with human vimentin, unexpected interactions can be exploited for biomedical application; intratumoral vascular imaging in this case [5]. Recently, it was found that the recombinant double-layer core particle of Bluetongue virus, that naturally infects ruminants, displays a human integrin-binding Arg-Gly-Asp (RGD) motif that may mediate efficient uptake by cancer cells [6]. However, rational modification to the exterior of VLNPs aims to use the strength of polyvalent interactions to engineer novel specificity for cell surface receptors. To this end, VLNPs have been functionalized with diverse ligands to tumor-associated targets such as peptides [7], antibodies [8], other proteins such as EGF [9], as well as glycans [10], aptamers [11] and small molecules such as folic acid [12]. These studies and others have shown remarkably efficient and specific *in vitro* targeting.

The type of cargo that VLNPs have been engineered to carry is equally as diverse as the range of targeting ligand. Similarly, there are many and varied methods by which cargos can be loaded. Depending on the specific VLNP, encapsidation can be achieved via the assembly of modified subunits with covalently linked cargo; co-assembly with noncovalently linked cargo interacting with subunits by programmed molecular interactions (e.g., protein–protein or RNA–protein); co-assembly with nonspecifically interacting cargo (e.g., electrostatic interactions or statistical encapsulation); templating assembly on inorganic particulate cargos; or via diffusion of small molecules through pores in the assembled VLNP shell. Taking advantage of the natural cargo of viruses, a number of VLNPs can be loaded with various nucleic acid based therapeutics, such as plasmids, mRNAs and siRNAs. Requiring more extensive bioengineering, the encapsidation of guest proteins is an exciting area with applications in advanced vaccine design [13], cytotoxic protein delivery [14] and genome editing [15]. Encapsidation of inorganic cargos such as quantum dots [16] and metallic cores offer opportunities in molecular imaging. A range of MRI and positron emission tomography agents have also been delivered by VLNPs [17]. Finally, VLNPs have been used to encapsidate high payloads of small molecule therapeutics such as doxorubicin [14], as well as photodynamic agents for light-triggered reactive oxygen generation [10,11].

It follows that the encapsidation of therapeutic or diagnostic cargos can be, and has been, combined with external functionalization in some of the examples previously mentioned [5,8,10,11,14,16]. The flexibility afforded by some VLNPs for dual modification means that both genetic and chemical modifications can be combined to create sophisticated, targeted nanoparticle delivery vehicles.

Challenges for VLNP-mediated therapeutic delivery

A recent study showed that the intrinsic immunostimulatory properties of Cowpea mosaic virus-derived VLNPs can have remarkable efficacy as an *in situ* cancer vaccine, generating a potent antitumor response in multiple tumor types [18]. However, while the inherent immunogenicity of virus capsids has driven the success of virus-like particle vaccines in the clinic as well as VLNP-based vaccine candidates [13,18], it remains a challenge to the application of therapeutic VLNPs in drug delivery. It is clear that the potential of VLNPs demonstrated *in vitro* will require greater understanding of VLNP interactions with the immune system if it is to be translated into *in vivo* outcomes. The pharmacodynamics and pharmacokinetics of VLNP delivery vehicles are beginning to be investigated by biodistribution studies [8,19] and these are providing valuable insight into the relationship between nanoparticles and heterologous biological environments. In addition, strategies from the biologics industry such as chemical modification with the neutral polymer, PEG, are being employed to prevent nonspecific uptake, escape immune surveillance and promote half-life of VLNP delivery vehicles [12,19]. Another approach demonstrated for VLNPs consists of decoration of particles with serum albumin to avoid immune detection [20]. Understanding *in vivo* fate

and strategies to reduce immunogenicity of VLNPs will be essential for their systemic application, as with any therapeutic vehicle.

It is likely that future therapeutics based on VLNPs will come from recombinant sources rather than productive infections. Given the lessons from gene therapies, the presence of nonspecific or, indeed, viral nucleic acid would vastly increase the regulatory burden. Interestingly, recombinant VLNPs can be structurally divergent from their cognate virion, which can provide opportunities for their further engineering [6]. The elucidation of VLNP structures and their development as delivery vehicles for therapeutics and imaging agents thus go hand-in-hand. Moreover, high-resolution structure determination by cryo-electron microscopy and single particle analyses, which is becoming the method of choice in structural virology, is facilitated by the symmetry, size and relatively straightforward purification techniques of VLNPs. Structural studies that combine or support the functional engineering of VLNP delivery vehicles are particularly informative and are likely to be more common as the field progresses.

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References

1 Lichty BD, Breitbach CJ, Stojdl DF, Bell JC. Going viral with cancer immunotherapy. *Nature Rev. Cancer* 14(8), 559–567 (2014).

2 Ungerechts G, Bossow S, Leuchs B *et al.* Moving oncolytic viruses into the clinic: clinical-grade production, purification, and characterization of diverse oncolytic viruses. *Mol. Ther. Methods Clin. Dev.* 3(Suppl. C), 16018 (2016).

3 Guenther CM, Kuypers BE, Lam MT, Robinson TM, Zhao J, Suh J. Synthetic virology: engineering viruses for gene delivery. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 6(6), 548–558 (2014).

4 Pokorski JK, Steinmetz NF. The art of engineering viral nanoparticles. *Mol. Pharm.* 8(1), 29–43 (2011).

5 Leong HS, Steinmetz NF, Ablack A *et al.* Intravital imaging of embryonic and tumor neovasculature using viral nanoparticles. *Nat. Protoc.*5(8), 1406–1417 (2010).

6 Brillault L, Jutras PV, Dashti N *et al.* Engineering recombinant virus-like nanoparticles from plants for cellular delivery. *ACS Nano.* 11(4), 3476–3484 (2017).

7 Hovlid ML, Steinmetz NF, Laufer B *et al.* Guiding plant virus particles to integrin-displaying cells. *Nanoscale* 4(12), 3698–3705 (2012).

8 Aanei IL, ElSohly AM, Farkas ME *et al.* Biodistribution of antibody-MS2 viral capsid conjugates in breast cancer models. *Molecular Pharm.* 13(11), 3764–3772 (2016).

9 Pokorski JK, Hovlid ML, Finn MG. Cell targeting with hybrid Qβ virus-like particles displaying epidermal growth factor. *Chembiochem.* 12(16), 2441–2447 (2011).

10 Rhee J-K, Baksh M, Nycholat C, Paulson JC, Kitagishi H, Finn MG. Glycan-targeted virus-like nanoparticles for photodynamic therapy. *Biomacromolecules* 13(8), 2333–2338 (2012).

11 Stephanopoulos N, Tong GJ, Hsiao SC, Francis MB. Dual-surface modified virus capsids for targeted delivery of photodynamic agents to cancer cells. *ACS Nano.* 4(10), 6014–6020 (2010).

12 Destito G, Yeh R, Rae CS, Finn MG, Manchester M. Folic acid-mediated targeting of cowpea mosaic virus particles to tumor cells. *Chem. Biol.* 14(10), 1152–1162 (2007).

13 Schwarz B, Morabito KM, Ruckwardt TJ *et al.* Virus like particles encapsidating respiratory syncytial virus M and M2 proteins induce robust T cell responses. *ACS Biomater. Sci. Eng.* 2(12), 2324–2332 (2016).

14 Ashley CE, Carnes EC, Phillips GK *et al.* Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles. *ACS Nano.* 5(7), 5729–5745 (2011).

15 Qazi S, Miettinen HM, Wilkinson RA, McCoy K, Douglas T, Wiedenheft B. Programmed self-assembly of an active P22-Cas9 nanocarrier system. *Mol. Pharm.* 13(3), 1191–1196 (2016).

16 Sun X, Li W, Zhang X *et al. In vivo* targeting and imaging of atherosclerosis using multifunctional virus-like particles of simian virus 40. *Nano Letters.* 16(10), 6164–6171 (2016).

17 Shukla S, Steinmetz NF. Virus-based nanomaterials as PET and MR contrast agents: from technology development to translational medicine. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 7(5), 708–721 (2015).

18 Lizotte PH, Wen AM, Sheen MR *et al. In situ* vaccination with cowpea mosaic virus nanoparticles suppresses metastatic cancer. *Nat. Nanotechnol.* 11(3), 295–303 (2016).

19 Bruckman MA, Randolph LN, VanMeter A *et al.* Biodistribution, pharmacokinetics, and blood compatibility of native and PEGylated tobacco mosaic virus nano-rods and -spheres in mice. *Virology* 449, 163–173 (2014).

20 Pitek AS, Jameson SA, Veliz FA, Shukla S, Steinmetz NF. Serum albumin 'camouflage' of plant virus based nanoparticles prevents their antibody recognition and enhances pharmacokinetics. *Biomaterials* 89, 89–97 (2016).