COMMENTARY

The hybrid AAVP tool gets an upgrade

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Gene therapy is the use of nucleic acids as therapeutic agents, with the purpose of restoring the expression of a missing/nonfunctional gene, silencing the expression of a mutant allele that has become toxic, or expressing cytotoxic genes to induce apoptosis and kill rogue cells. The last of the three is the main strategy used for cancer gene therapy. One of the major hurdles of targeting solid tumors for clinical translation, however, is the lack of delivery vehicles capable of inducing a robust expression of the transgene in the target population of cells while keeping immunogenicity and off-target effects to a minimum. While mammalian viruses have been the most widely used due to their natural ability to express foreign DNA or RNA in the nucleus of infected cells, several publications have demonstrated that hybrid prokaryotic–eukaryotic viral vectors can be attractive vehicles, particularly for genetic-mediated imaging and therapeutic intervention in tumor cells (1). The hybrid adeno-associated virus (AAV)/phage (AAVP) has been successfully used in several in vivo models to target solid tumors with greater specificity, facilitating positron emission tomography (PET) imaging and suppressing tumor growth by disrupting tumor angiogenesis. In PNAS, Suwan et al. (2) increase the versatility of AAVP by designing 2 phages capable of avoiding nonspecific adsorption and escaping endosomal-mediated degradation, significantly improving the transduction outcome. This work not only will have important implications for phage-mediated cancer gene therapy but may also facilitate similar optimizations in other gene transfer vectors.

Over millions of years, viruses have evolved strategies to introduce their viral genome into target cells, subsequently hijacking the host's cellular machinery to replicate. In recent years, this has made them very attractive for gene therapy applications, but the choice between prokaryotic phage and mammalian viruses needs to include careful consideration of each vector's advantages and disadvantages and their compatibility for a given application. Mammalian viruses can be potent delivery vehicles for human cells and are widely used in clinical trials worldwide, but their in vivo use often results in nonspecific tissue transduction due to their native tropism and production costs can be considerably high. On the other hand, prokaryotic phages have evolved to specifically infect bacteria and have no native tropism for mammalian targets, meaning surface ligands can be adapted and engineered to bind particular mammalian receptors, targeting them to certain cell populations with great specificity. Their use is considered safe in humans, and their production method makes it cheaper for clinical translation (3). This makes phage-based vector systems attractive for cancer gene therapy, where specificity is of the utmost importance. However, basic phages also lack the necessary tools required for efficient transgene expression in mammalian cells and are therefore considered poor delivery vehicles.

To overcome the phages' poor eukaryotic transduction profile, Hajitou et al. (1) first developed the hybrid virus AAVP over 10 y ago, consisting of a phage coating structure capable of delivering an inverted terminal repeats-flanked AAV transgene, which conferred greater stability to the construct upon transduction and enhanced its expression efficiency. Using a ligand that targets the tumor vasculature (RGD-4C) inserted in the phage's minor pIII coating proteins, Hajitou et al. (1) demonstrated how systemic administration of the RGD-4C AAVP selectively targeted tumors in vivo. This system then allows the transfer of the AAV transgene of choice, enabling bioluminescence imaging and PET imaging and even inhibiting tumor growth, depending on content of the transgene (1). Despite these advances, the transduction efficiency of target cells remained low when compared with some mammalian viruses, mainly due to antibody neutralization and nonspecific adsorption by charged molecules before transduction and endosomal-mediated degradation of the viral particles once inside the cells (Fig. 1*A*).

In PNAS, Suwan et al. (2) overcome these obstacles by incorporating specific peptide sequences in the

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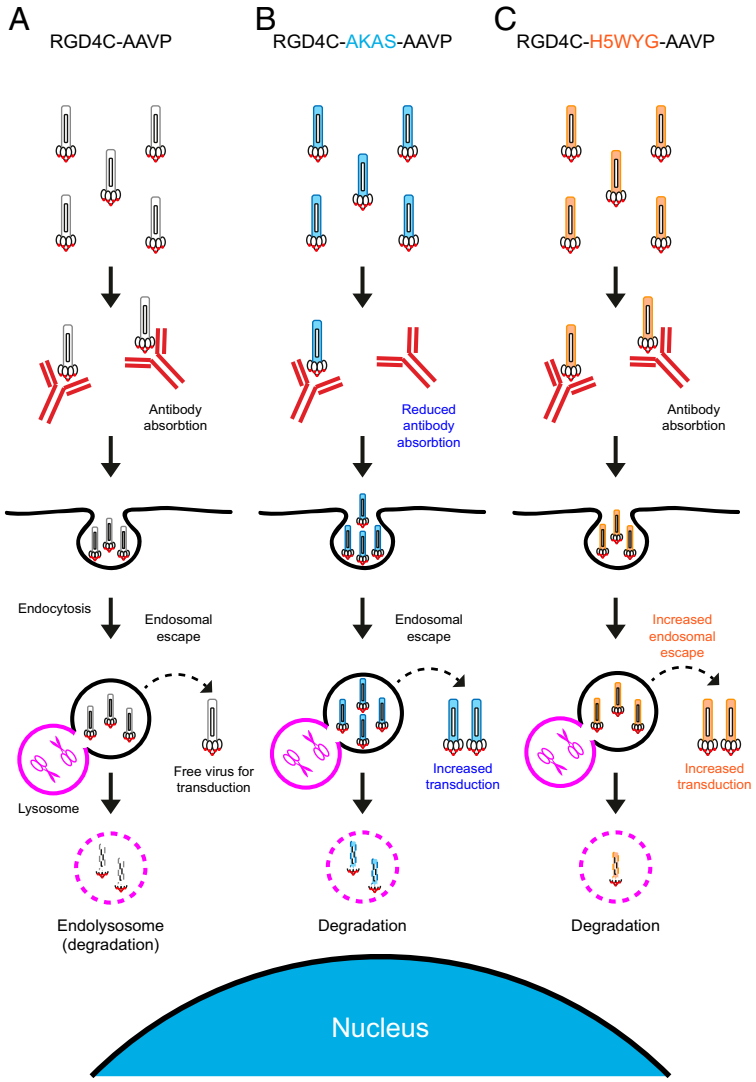

Fig. 1. Next-generation AAVP phages can overcome pre- and postinternalization barriers to transduction. (*A*) The original RGD4C-AAVP can transduce mammalian cells, although its efficiency is reduced due to antibody neutralization and endosomal-mediated degradation. (*B*) Introduction of the AKAS peptide into the pVIII phage coating reduces nonspecific adsorption and neutralization by antibodies, increasing the transduction efficiency. (*C*) When expressed in the rpVIII coat protein, the histidine-rich H5WYG peptide promotes endosomal escape of the AAVP, decreasing endosomal-mediated degradation and enhancing transduction.

major pVIII coat proteins to confer degradation-resistance properties to the phages, while maintaining the RGD-4C targeting ligand on the pIII minor coat protein, to direct these next-generation particles to solid tumors cells. After demonstrating that these peptides do not compromise the functionality of the phage or its ability to transduce cells, Suwan et al. (2) introduced the charged neutralizing sequence Ala-Lys-Ala-Ser (AKAS) in the wild-type pVIII coating, conferring zwitterionic properties to the virus. By incorporating this AKAS neutralizing peptide, the overall charge of the phage was shifted toward neutrality, significantly decreasing the nonspecific adsorption by fibrinogen and promoting its escape from a neutralizing antibody, resulting in enhanced tumor cell transduction (Fig. 1*B*).

Upon entry of the viral particles into the intracellular space, endosomal-mediated degradation poses another significant barrier to efficient transduction. To create enhanced phages with the ability to bypass this obstacle, Suwan et al. (2) selected 3 different peptide sequences that could promote endosomal escape and introduced them in the rpVIII gene. The histidine-rich H5WYG peptide was found to confer buffering properties to the phage while encapsulated in endosomes, likely resulting in increased osmotic swelling of the endosome, destabilization, and release of the phages to the cytoplasm (Fig. 1*C*). This next-generation RGD4C-H5WYG-AAVP containing the luciferase reporter gene was shown to have markedly improved transduction efficiency at different viral concentrations when compared with phages without this peptide and was able to promote targeted gene delivery in cancer cell lines. Importantly, Suwan et al. (2) utilized bafilomycin A1, an inhibitor of the vacuolar ATPase proton pump, to prevent endosomal protonation, and convincingly show that the increased transduction efficiency is indeed due to endosomal escape.

Suwan et al. (2) also show that RGD4C-H5WYG-AAVP was able to express luciferase in tumors in vivo and did so more efficiently than standard RGD4C-AAVP, with up to 3.5-fold higher expression. In the future, it will be interesting to see how this enhanced targeted gene delivery can be weaponized to inhibit tumor growth or to facilitate PET imaging applications. Finally, since Suwan et al. (2) demonstrate that a multifunctional hybrid AAVP

with both a wild-type and a recombinant pVIII is viable, a single phage expressing both the AKAS neutralizing peptide and the H5WYG endosomal escape peptide becomes theoretically possible and, if functional, could potentially combine the enhancing properties of both peptides.

These studies by Suwan et al. (2) improve the versatility and power of the AAVP tool, but they also hold promise for optimization of other vectors, with even broader applications. Indeed, antibody-mediated neutralization and endosomal and ubiquitin–proteasome-mediated degradation are also key barriers for transduction by AAV viruses, one of the most widely used gene therapy vehicles for noncancer applications (4–6). Strategies to overcome this have included mutagenesis of specific regions of the capsid (7, 8) or the use of capsid decoys combined with therapeutic AAV (9). One can hypothesize that the modifications reported by Suwan et al. (2) might be suitable candidates for similar optimizations in AAV, since their efficacy has already been established in a viral transduction setting. Of course, caution will be needed to ensure the immunogenicity of any such engineered viruses remains low, but these studies open up the possibility of engineering viral strains with a narrower tissue tropism, reduced off targets, and therefore more specific and efficient gene delivery.

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