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Hypothesis

Encapsulated Bacteria with a Light-Repressed Deadman Switch for Liver Gene Delivery

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Abstract: Adeno-associated viruses (AAVs) have been used for liver gene therapy. Hemgenix and Roctavian are AAV-based treatments for hemophilia B and A, respectively. They cost $3 million and $2.8 million per dose, respectively. While AAV vectors may eventually be cheaper to mass produce, a bacteria-based DNA delivery system might be much cheaper for patients. Also, "bactofection" would allow for the delivery of much larger DNA packages. Such a bacterial system may now be possible, and a prototype for the liver could possibly be developed immediately.

Keywords: encapsulation; adeno-associated virus (AAV); light-activated allosteric protein switch; Deadman switch; T4 bacteriophage gene delivery vector; and facultative intracellular bacteria

1. Introduction

Adeno-associated viruses (AAVs) can only encode ~5 kb of DNA maximum, which is not sufficient to cure certain individuals with genetic disorders wherein long stretches of nucleotides are affected, e.g., at least some cases of Duchenne muscular dystrophy [1–6].

Apparently, "…in principle prime editing can correct up to ~89% of the 75,122 pathogenic human genetic variants in ClinVar…" [7]. New prime editing-based techniques can almost surely cover even more variants [8]. However, multiple proteins/RNAs/DNA molecules (or at least a single, large DNA molecule) may be required in certain cases when more complex molecular machinery must be employed - e.g., if heterochromatin is an issue.

Also, the cost of producing sufficiently high titers of AAV vectors and other viral vectors for therapeutic purposes is very steep [9–11].

Intravenously injected lipid nanoparticle (LNP)-encapsulated CRISPR ribonucleoproteins (RNPs) have been successfully utilized in the liver and somewhat in the lungs [12,13]. However, current LNP formulations at least cannot effectively reach or target cells in the CNS, heart, or kidneys after intravenous injection [13,14]. Also, the larger the LNP - the less effective it is at extravasation. Thus, it cannot encapsulate large cargos unless targeted to the liver or spleen. Also, LNP production costs are still substantial - and scaling up the production of new ionizable lipids is not that facile [15–18].

Intrathecal and intracerebroventricular administration of RNPs, as well as direct intraparenchymal injection of RNPs into the striatum, does not result in widespread CNS editing [19].

A synthetic bacterial vector with low immunogenicity that only delivers to particular organs or organ systems would be of use for multiple reasons.

1. It would be cheap.
2. It could deliver large DNA constructs to use as homologous repair templates or large serine recombinase/CRISPR transposase cargo for individuals with genetic disorders wherein long stretches of nucleotides are affected.
3. May be able to extravasate at least moderately efficiently in most anatomical locales - and then specifically proliferate in the target organ or organ system [20,21]. (Worst case scenario - intraparenchymal injections could be employed.)
4. Flagellar motility of the vector allows for autonomous, widespread delivery throughout the organ or organ system [22].

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2. Prototype for the Liver

The *Escherichia Col*i Nissle 1917 or *Salmonella* Typhimurium vector could be encapsulated [23] to prevent cytotoxic protein secretion from affecting the tissue environment and substantially attenuate the immune response. It can also be myristoylation negative [24]. One could employ an anti-sepsis small molecule - at least in a mouse model [25]. Dexamethasone would be used to further suppress the immune response and induce the expression of CY3PA in the liver. One would then administer a caged luciferin molecule that is uncaged by CY3PA [26].

The bacteria would express firefly luciferase [27,28] and have a Deadman switch that is sensitive to (blue-shifted) light [29,31]. Bacteria that stray outside of the liver - where the luciferin is uncaged - will quickly (stop replicating and) lyse.

When the bacteria in the liver reach a high enough population level (either visualized with an MRI-based reporter gene [32] or just after an experimentally-determined period of time), they can be induced via small molecule to produce a large quantity of CRISPR RNPs [19,33] and lyse.

Theoretically, E. coli Nissle 1917 is endotoxin-free. However, to prevent possible issues from mass lysis, perhaps the small molecule should stop motility immediately - while the gene circuit for lysis can be noisy [34].

Another option would be to use a second Deadman switch; a molecule that the bacteria are exposed to in solution prior to intravenous injection would be gone after injection, and eventually they will just (stop replicating and) lyse naturally.

To deliver DNA and RNA, the bacteria could instead produce large quantities of an engineered T4 bacteriophage [35]. However, this phage is not very efficient at entering eukaryotic cells - or at least escaping the endosome.

Thus, instead of just lysing - after small molecule administration or the second Deadman switch time period ending - it could lose the capsule, enter hepatocytes [36], escape the endosome if necessary [37], overproduce an engineered T4 phage, and lyse [37].

3. Other Organs and Organ Systems

We could identify other enzymes specific to an organ or organ system and develop caged luciferins for those as well. This system does not have to be caged luciferin/luciferin, though, of course. It could be an organ/organ system-specific extracellular protease, for example. This protease could activate regulated intramembrane proteolysis [38] or cleave a pro-peptide that activates a two-component regulatory system [39]. The benefit of such an approach would be that the bacteria themselves would secrete the molecule required for their continued survival - which would be locally activated in the organ or organ system of choice. This might not produce a sufficiently intense signal, though.

Bacteria can also cross the blood-brain barrier after intravenous injection [21,40].

There is a caged luciferin that can be uncaged in the brain - but it also is uncaged in the kidneys [41]. A better strategy - for now at least - would just be to administer a small molecule that is blood-brain barrier-impermeable and that kills any of the synthetic bacteria in the periphery.

In a paper by Dr. Antas et al., it was said that "Although nonviral NPs may tackle the main limitations of their viral counterparts, they have thus far failed to compete with the transduction efficiency achieved by viral vectors in the retina" [42].

Bactofection could be better than AAVs for retinal conditions. A Deadman switch could be used to only allow (limited) bacterial replication in the eye via a small molecule present in an eye-drop solution.

Identifying tissue-specific enzymes should not be very difficult nowadays [43].

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