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Non-viral gene delivery

Multifunctional polyplexes as locally triggerable nonviral vectors

JA Hubbell

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If drugs consist of a formulation of a bioactive and a carrier or vector, historically the lion's share of the sophistication has been attributed to be in the bioactive, the carrier or vector being considered rather the boring part. When the carrier is merely saline with a few simple additives, perhaps this perspective might be accurate, but with many modern biomolecular drugs, including DNA, the carrier may account for as much of the glory of the formulation as the bioactive itself, the vector critically enabling the biomolecule to carry out its designed biological function. This certainly is the case with a new non-viral gene delivery vector described by Kazunori Kataoka and colleagues in a recent paper in Nature Materials, in which they describe molecular machines that bind DNA and assist its delivery to cells, hop from the DNA to the endosomal membrane after endocytosis, and then interact with light to destabilize the membrane, thus enabling endosomal escape of the DNA.[1]

With non-viral vectors for delivery of DNA, several highly functional polymers have been explored to package DNA into nanoparticles, protect it from degradation and enhance its binding to cells and transport into the cytoplasm. The broadly used polycation polyethylene imine (PEI), popularized by Jean-Paul Behr at the Université Louis Pasteur in Strasbourg, France, both condenses the DNA by counter charge complexation and helps to destabilize the endosome via an osmotic imbalance created by the so-called proton sponge effect, in which more and more ions are pumped into the endosome in an effort to acidify it against the large backdrop of the basic polymeric vector.[2] Kevin Rice, at the University of Iowa, developed a still more functional polycation, in an effort to counter the toxicity that can be associated with these highly positively charged macromolecules, by designing short positively charged peptides that crosslink via disulfide bonding into stable peptide–DNA polyplexes outside the cell but fall apart into the parent less toxic shorter peptides in the reductive environment of the endosome.[3] Mark Davis, at the California Institute of Technology and his colleagues at Insert Therapeutics in Pasadena, California, developed another approach to functional polycations, consisting of cationic polymers with cyclodextrin rings within them, the cationic nature enabling DNA complexation and the cyclodextrins hosting binding of polyethylene glycol-grafted cell targeting groups to the nanoparticle.[4] Jean Fréchet at the University of California at Berkeley has developed approaches not using polycations at all, by entrapping DNA within crosslinked hydrogel microparticles that are stable in the extracellular environment but are triggered to quickly degrade and release the DNA upon acidification of the endosome.[5]

One of the more serious needs for functionality in vector design is to effectively overcome the barrier to cytoplasmic entry presented by the endosomal membrane. This, of course, is swimming a bit upstream, as the endosome has evolved to move quite stably through the cell, carrying a variety of biomolecular payloads to different intracellular destinations, including the here unwanted lysosome for destruction of the contents, but release of the endosomal contents to the cytoplasm was apparently not physiologically considered. Toward the end of enhancing endosomal escape, Allan Hoffman and Patrick Stayton at the University of Washington along with David Tirrell at the California Institute of Technology developed a water-soluble polymer that displays no activity at extracellular pH but binds to biological membranes and becomes highly membranolytic during endosomal acidification.[6] Using such approaches, it is possible to enhance delivery of DNA into the cytoplasm after endosomal uptake.

Other areas of medicine also use membrane disruption for beneficial outcomes. One notable approach is photodynamic therapy, in which a light-absorbing sensitizer is delivered to the cell.[7] Upon irradiation with light, the sensitizer is excited to a singlet state, converts to a triplet state and then reacts with oxygen to form singlet oxygen. Singlet oxygen is highly reactive and can oxidize unsaturated fatty acids found in the cell's membranes, creating points of instability that then aggregate to a larger defect, leading to perforation of the membrane.[8] In photodynamic therapy, the target is the plasma membrane, and the desired outcome is induction of cell death. Kristian Berg at the Norwegian Radium Hospital has developed an approach by which these phenomena can be harnessed to disrupt the endosomal membrane, rather than the plasma membrane. This process, referred to as photochemical internalization, has been used to enhance transfection using both non-viral and viral vectors.[9–11]

In a recent paper by Kazunori Kataoka and colleagues at the University of Tokyo, many of the above concepts of biological functionality were combined to a very high level of sophistication in vector design.[1] Firstly, DNA was complexed using a disulfide-extended cationic peptide, the sequence of which was designed based on the HIV-1 TAT protein, which may further assist in escape of the DNA from the endosomal membrane and in nuclear localization.[12] Secondly, the cationic peptide–DNA polyplex formed from the above was exposed to a negatively charged dendrimer, containing a photosensitizer at the core and 32 carboxyl groups at the surface. When the polyplexes are taken up into the endosome, and endosomal acidification proceeds, the dendrimer becomes less negatively charged and is presumed to dissociate from the peptide–DNA polyplex. When it becomes less charged, it also becomes more hydrophobic, which means that it may associate with the endosomal membrane much

better. The most effective agents in photochemical internalization also associate with endosomal membranes.[13] This is particularly important, as singlet oxygen is highly reactive and acts within 10–20 nm of its formation.[14] As such, it may be especially important that the dendrimer encapsulating the photosensitizer dissociates from the DNA and moves to the endosomal membrane, minimizing damage to the DNA and maximizing disruption of the endosomal barrier. Using this approach, Kataoka and colleagues demonstrated that it was possible to enhance transgene expression more than 100-fold with light irradiation. Moreover, the observed efficient gene transfer was associated with only a 20% decrease in cell viability, whereas controls with PEI transfection showed an 85% decrease in viability. The most unique feature of the photochemical internalization approach developed by Berg and now extended by Kataoka is the ability to localize gene transfer to the site of irradiation. Indeed, when this novel vector was tested in gene transfer in the conjunctiva in the rat, transgene expression was observed only in the irradiated zone.

Non-viral vectors for DNA transfer are making substantial strides by employing a number of clever approaches in functional polymer design. The approach by Kataoka and colleagues involves a number of such functionalities – complexation, shifting of an active molecule from the polyplex to the endosomal membrane and endosomal disruption and escape – to achieve efficient, nontoxic and localizable gene transfer. ■

JA Hubbell is at the Institute of Bioengineering at the École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.
E-mail: jeffrey.hubbell@epfl.ch
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