Nanotechnology for organ-tunable gene editing

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TARGETED GENETIC DRUG DELIVERY

Nanotechnology for organ-tunable gene editing

Lipid nanoparticles containing genetic drugs can be bioengineered to tune their biodistribution and induce organ-specific gene regulation.

Roy van der Meel

Lipid nanoparticle (LNP) technology has enabled the clinical translation and first-ever Food and Drug Administration (FDA) approval of a small interfering siRNA (siRNA) drug¹. This nanodrug, developed for treating polyneuropathies resulting from the hereditary disease transthyretin-mediated amyloidosis¹, relies on efficient LNP-mediated siRNA delivery to hepatocytes following intravenous infusion, resulting in subsequent inhibition of pathological protein production. LNP systems also allow encapsulation of messenger RNA (mRNA) to induce therapeutic protein production² and of gene editing complexes to correct disease-causing mutations³ in hepatocytes following systemic administration. However, achieving therapeutically relevant gene modulation in tissues and organs other than the liver remains elusive thus far.

In this issue of *Nature Nanotechnology*, Qiang Cheng et al. present an approach called Selective Organ Targeting (SORT), whereby LNPs containing nucleic acid-based therapeutics are bioengineered to induce liver-, spleen- and lung-specific gene regulation⁴. LNP systems typically consist of phospholipids, cholesterol, polyethylene glycol (PEG)-lipids and ionizable cationic lipids. Each LNP component and their molar ratios have previously been optimized to ensure efficient nucleic acid delivery to hepatocytes⁵. In particular, rational design and screening of ionizable cationic lipid (or lipid-like) libraries has been essential for achieving potent hepatic gene silencing at doses relevant for clinical applications^{6,7}. The most effective ionizable cationic lipids, characterized by an apparent pK_a value between 6.2 and 6.5 (ref. ⁸), fulfill three functions: (1) at acidic pH, the tertiary amines in the lipid head groups become protonated to promote efficient complexation with negatively charged nucleic acids during LNP production, (2) at physiological pH, the uncharged lipids ensure a near net-neutral surface charge of LNPs to reduce immune responses and to prolong circulation time and (3) in the acidified endosome following cellular

Fig. 1 | Organ-tunable therapeutic gene modulation using engineered lipid nanoparticles. SORT-LNPs are engineered by rapid mixing procedures. Reporter models based on luciferase expression and a Cre-Lox approach coupled to td-Tomato (tdTom) fluorescence were used to identify SORT-LNPs for tissue-specific gene expression and editing. hEPO, human erythropoietin; IL-10, interleukin 10. Detailed schematic of LNP adapted with permission from ref. ¹², American Chemical Society.

uptake, the positive charge of the lipids promotes membrane fusion and cytosolic payload delivery.

In the SORT approach, the authors added a fifth lipid component to LNPs for delivery of functional mRNA or gene editing

complexes to specific tissues following intravenous administration in mice (Fig. 1). Using a rapid mixing procedure, the SORT lipid type (permanent cationic and anionic or ionizable cationic) and molar percentages were systematically varied to generate LNPs containing mRNA encoding luciferase for in vivo screening. By adding increasing molar percentages (0–100%) of the permanently cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), luciferase expression could be shifted from liver to spleen to lungs after intravenous injection. Equipping LNPs with 10–40% of the permanently anionic lipid 1,2-dioleoyl-sn-glycero-3-phosphate (18PA) resulted in luciferase expression exclusive in the spleen. Adding 20% of other ionizable cationic lipids such as 1,2-dioleoyl-3-dimethylammonium-propane (DODAP) did not alter biodistribution but enhanced mRNA delivery to the liver. The universality of the approach was confirmed by functionalizing LNP formulations with other permanently charged or ionizable cationic lipids, which led to similar changes in organ expression in a lipid-class-dependent manner. Of note, ionizable cationic lipid inclusion was indispensable for the SORT-LNP efficacy. Administering lung-, spleen- and liver-specific mRNA SORT-LNP formulations identified from the luciferase-based screen resulted in sustained therapeutic protein production without significant toxicity. Importantly, the SORT approach also allowed the tuning of tissue-specific luciferase expression for LNPs identical to the clinically approved formulation Onpatro.

Cheng et al. also report tissue-specific CRISPR/Cas-based gene editing using SORT-LNPs. In contrast to therapeutic

gene silencing or expression, CRISPR/Cas-based gene editing requires (at least) two components: guide RNA (gRNA) to recognize target DNA and Cas nuclease to make a double-strand break. The authors generated SORT-LNPs containing gRNA and Cas9 mRNA or gRNA/Cas9 ribonucleoprotein complexes and demonstrated gene editing in extrahepatic tissues. In red fluorescent protein tdTomato reporter mice, different SORT LNP formulation types allowed selective induction of liver-, lung- or spleen-specific gene editing. Moreover, the authors show tissue-specific editing of an endogenous target gene (*PTEN*) and a therapeutic target gene (*PCSK9*).

The main innovation of the study by Cheng et al. revolves around the systematic LNP screening approach in sophisticated reporter models⁹. A similar approach, called Fast Identification of Nanoparticle Delivery (FIND), was reported by Sago et al.¹⁰. Their strategy included screening hundreds of LNPs to identify potent formulations for delivering functional mRNA (and other RNA therapeutics) to various tissues using rationally designed DNA barcodes.

The premise that LNP technology can be straightforwardly engineered to enable therapeutic gene regulation in specific tissues (and cells) is exciting as it would potentially enable organ-specific gene therapies for the treatment of, for example, cystic fibrosis (lung), lymphomas (spleen) or immune disorders. Although the authors speculate about altered apparent pK_a values and distinct protein coronas, precise mechanistic studies are needed that reveal how SORT modification redirects LNPs to specific tissues. Along these lines, it was previously reported that

adjusting the negative net charge of mRNA lipoplexes governs luciferase expression in tissue-specific antigen presenting cells. Systemically administering positively-charged mRNA lipoplexes resulted in luciferase expression predominantly in the lungs, while decreasing the cationic lipid content shifted expression to the spleen¹¹. Improved mechanistical insight will enable further fine-tuning of LNP organ- and tissue-specificity. However, possible immunogenicity and infusion-related effects need to be thoroughly investigated before the SORT approach can be implemented to rationally design translational LNP formulations for therapeutic gene modification of organs other than the liver. □

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