Drug delivery

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Inhalable mRNA nanoparticles

Ronnie H. Fang & Liangfang Zhang

A large-scale screening identifies an inhalable polymer nanoparticle formulation that safely and effectively delivers therapeutic mRNA molecules to the lungs of several animal species.

The COVID-19 pandemic has highlighted the potential of mRNA-based medicine for vaccine applications[1]. In principle, any type of protein can be expressed using mRNA technology, thus making it broadly applicable for the prevention and treatment of many diseases[2]. Despite their immense promise, mRNA molecules are fragile, and it is difficult for them to get inside cells, where they can engage with the cellular machinery that is responsible for converting their encoded information into proteins. To overcome these challenges, mRNA molecules can be encapsulated in nanoparticle carriers that offer protection from degradation by enzymes while enhancing cellular entry[3]. Current mRNA-based nanovaccines being used in the clinic to battle severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are administered intramuscularly; however, they are ineffective when delivered to the lungs via inhalation, which is a highly desirable route of administration due to its relative simplicity and potential for improved patient compliance. Now, writing in *Nature Materials*, Rotolo and co-authors[4] report on mRNA polymeric nanoparticles that can be safely delivered to the lungs of animals of various species using a nebulizer to elevate the local production of therapeutic proteins (Fig. 1).

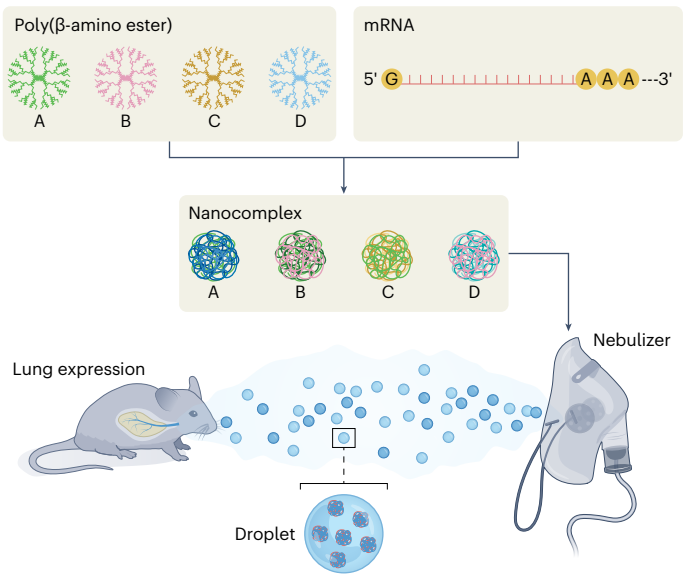

Fig. 1 | Screening of mRNA nanocomplexes for protein expression in the lungs. An mRNA payload is combined with a library of poly(β-amino esters) to form mRNA nanocomplexes. The activity of each nanocomplex is then screened in vivo by delivering it to the lungs via nebulization. Figure adapted with permission from ref. [4], Springer Nature Ltd.

To create the mRNA nanoformulation, a library of 166 different polymers based on a previously reported hyperbranched poly(β-amino ester) was constructed. Each of the polymers containing various modifications was combined with mRNA encoding for a protein construct. The resulting nanocomplexes were then evaluated for their ability to transfect the lungs of mice. To facilitate the in vivo screening process, the authors employed a nebulizer setup with a low dead-volume that reduced the amount of sample required for each experiment. From the initial screen, five promising formulations were identified based on their efficiency in delivering mRNA to the lungs, and finally a single lead polymer candidate containing a dithiol group, identified as P76, was selected.

Using the P76 polymer, mRNA of increasing sizes encoding for a variety of proteins was successfully formulated and delivered to the lungs of mice for protein expression. These data provided a strong indication that the platform could be employed for 'plug-and-play' mRNA delivery, where the mRNA payload can be easily swapped for another one depending on the desired application. Next, a comprehensive set of safety studies was performed, demonstrating that the nanoparticle formulation does not induce any toxicity when delivered to the lungs. Further validation studies in hamsters, ferrets, cows and monkeys confirmed the efficiency of this polymer formulation in delivering mRNA to the lungs of different animals. mRNA nanocomplexes produced using the P76 polymer were well tolerated in all animal species, while also improving in vivo protein production compared with formulations made using a previously reported poly(β-amino ester) polymer.

To evaluate the performance of the platform in a clinically relevant disease setting, a hamster model of SARS-CoV-2 infection was employed. As a treatment, the animals were administered a P76 nanocomplex loaded with RNA molecules for producing a Cas13a-based CRISPR complex against the SARS-CoV-2 nucleocapsid protein (an important structural component of the virus). Treatment with the nanoformulation protected the hamsters against the ill effects of SARS-CoV-2 infection, resulting in healthy weight gains over time in comparison to untreated animals and those that were treated with the same RNA but complexed with a control poly(β-amino ester) polymer. On a per milligram basis, the RNA-based treatment also outperformed the systemic administration of a virus-neutralizing antibody, which the authors employed as a gold-standard control treatment.

The work of Rotolo and co-authors highlights the advantages of performing large screens to identify promising nanomaterials that can be used for different biomedical applications. In this case, a highly functional polymer for effective mRNA delivery to the lungs was successfully identified, and this could have important implications for the treatment of various lung-related pathologies. For example, cystic fibrosis is characterized by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene[5], and thus an mRNA-based

approach for producing the correct version of the corresponding protein within the lungs may help to correct the disease phenotype. In contrast to systemic delivery approaches, being able to localize mRNA payloads to the desired site of action can greatly improve their therapeutic index. The high in vivo transfection efficiency afforded by the newly identified P76 polymer also enabled considerable dose sparing, which is of critical importance for future clinical translation. While the potency of the P76-based mRNA nanocomplexes was validated in multiple animal models, the true test will come when evaluating in human patients. Along these lines, further studies to elucidate the precise mechanism behind the improved transfection may provide additional confidence that the platform will seamlessly translate into humans. Overall, mRNA-based medicines have proven that they have much to offer, and the continued development of technologies to facilitate their clinical application will be critical in ensuring that they reach their full potential.

Ronnie H. Fang [1,2] **& Liangfang Zhang** [1,2] ✉
[1]Department of NanoEngineering and Chemical Engineering Program, University of California San Diego, La Jolla, CA, USA. [2]Moores Cancer Center, University of California San Diego, La Jolla, CA, USA.
✉e-mail: zhang@ucsd.edu

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Competing interests

The authors declare no competing interests.