Development and evaluation of localized mRNA delivery systems

for vaccines and inhaled therapies

By

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Messenger RNA (mRNA) represents a promising new class of drugs that can be utilized not only as vaccines for infectious diseases and oncology, but also as protein replacement and gene editing therapies for the treatment of genetic diseases. However, one of the major challenges for the clinical translation of mRNA drugs is safe and effective delivery of the mRNA to target tissues and cells. For mRNA vaccines, another challenge is to carefully control immune stimulation to enhance productive immune responses to the encoded antigen while minimizing reactogenic side effects. In this thesis, we address these challenges by developing localized mRNA delivery systems to improve the safety and efficacy of vaccines and inhaled therapies.

We first developed lipid nanoparticle (LNP) formulations which enabled effective, localized delivery of mRNA to the lung epithelium following nebulized administration. Nebulized mRNA-LNPs face several unique challenges including stability during nebulization and penetration through both cellular and extracellular barriers. Through design-of-experiment formulation screening and rational design of excipients and buffer conditions, LNPs were stabilized to nebulization which resulted in significantly improved inhaled mRNA delivery in the lungs of mice. We combined the stabilized LNP nebulization formulation with novel, biodegradable ionizable lipids screened using air-liquid interface cultures to achieve improved mRNA delivery to the lungs over state-of-the-art LNPs and polymer nanoparticles. Additionally, we optimized nebulized LNPs through PEG-alternative polymer modifications to LNP surfaces, resulting in LNPs with greater stability, mucus diffusivity, and transfection efficiency in both healthy and diseased lung models.

While mRNA vaccines have proven highly effective against COVID-19, opportunities remain to develop more potent vaccines which elicit improved immune responses to the target antigen while limiting reactogenic side effects. To achieve this, we optimized the vaccine by screening a library of 480 biodegradable ionizable lipids with headgroups adjuvanted with cyclic amines and by adjuvanting the mRNA-encoded antigen by fusing it with a natural adjuvant derived from the C3 complement protein, C3d. In mice, intramuscular or intranasal administration of LNPs with the lead ionizable lipid and with mRNA encoding for the fusion protein (either the spike protein or the receptor-binding domain of SARS-CoV-2 fused to C3d) increased the titers of antibodies against SARS-CoV-2 tenfold compared to an unadjuvanted vaccine. The multi-adjuvanted mRNA vaccines presented here may improve the efficacy, safety and ease of administration of mRNA-based immunization.

Taken together, the work presented in this thesis contributes to the field of mRNA therapeutics by introducing new, combinatorial screening and rational design approaches towards improving the safety and efficacy of mRNA vaccines and inhaled mRNA therapies.

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