Nucleoside Base Modifications Improve Mrna Delivery In A Carrier-dependent Manner

Jilian Melamed
Carnegie Mellon University

Abstract

Introduction: Gene therapy with mRNA has outstanding clinical potential to transform disease management. Two challenges have precluded the clinical translation of mRNA therapies: 1) achieving potent and specific mRNA delivery to target cells, and 2) evading immunogenicity. These challenges can be addressed by rationally engineering both the mRNA payload and the carrier. Chemical mRNA base modifications can improve both the potency and immune evasion of mRNA. Here, we ask if a specific base modification is always best, or if modifications need to be tailored to different carriers.

Methods: We tested mRNAs with five commonly used base modifications (Fig 1A) formulated into four different lipid nanoparticles that deliver mRNA to different organs (Fig 1B-E). Particles were delivered to mice, and *in vivo* imaging quantified mRNA delivery to different organs. Using flow cytometry, we further investigated which cell types undergo the greatest improvement in mRNA delivery with modified nucleobases.

Results: For all nanoparticles, the m1Ψ nucleoside modification provided the greatest increase in translation efficacy. Interestingly, the degree of this improvement occurred in an organ- and lipid-dependent manner (Fig 2). We found that Ψ, m1Ψ, and m5C/Ψ-modified mRNAs underwent the greatest translation enhancement in the spleen, relative to unmodified mRNA. This occurred predominantly in carriers that naturally deliver to the spleen, while only modest improvements in splenic translation were observed for m1Ψ- and m5C/Ψ-mRNA with the liver-targeting carrier cKK-E12. Interestingly, no modifications improved lung delivery using the lung-targeting material, ZA3-Ep10. At maximum, C12-200 carrying m1Ψ-modified mRNA achieved a massive 54-fold increase in efficacy relative to unmodified mRNA in the spleen, whereas this delivery combination increased translation in the liver only 6-fold. We further found that splenic translation enhancement is the combined result of increased numbers of transfected cells and increased translation per cell. Surprisingly, the m1Ψ modification did not improve delivery to lymphocytes, which constitute 65-90% of splenocytes. Instead, m1Ψ-mRNA improved C12-200-mediated delivery to monocytes, macrophages, neutrophils, and dendritic cells. Interestingly, m1Ψ-mRNA decreased $200O_{i10}$-mediated delivery to these cells, underscoring the importance of choosing modifications appropriately for the targeted population.

Conclusion/Implications: Selecting the appropriate mRNA base modification is highly dependent upon the carrier in use and the desired organ target. Our results provide important guidelines for the rational design of targeted mRNA therapies.

Acknowledgements: This research was supported by the Curci Foundation and DARPA Grant No. D16AP00143.
References: 1. Hajj, K. A.; Whitehead, K. A. Nat. Rev. Mater. 2017, 2, 1–17.2. Kariko, K.; Weissman, D. Current Opinion in Drug Discovery Development 2007, 10 (5), 523–532.3. Kariko, K.; Buckstein, M.; Ni, H.; Weissman, D. Immunity 2005, 23 (2), 165–175.
Keywords: Type of delivery agent - DNA/RNA, Delivery vehicle - Nanoparticle/nanomaterial, Delivery vehicle - Rational design, Focus groups - Nanomedicine and Nanoscale Drug Delivery (NND), Focus groups - Gene Delivery and Gene Editing (GDGE), Delivery vehicle - Liposome/micelle/suspension
Learning Objectives:

- Recognize that mRNA therapies need to be rationally designed by considering both the carrier and the payload.
- Demonstrate that nucleobase modifications generally improve mRNA delivery, but to the greatest extent in the spleen.
- Understand that mRNA base modifications exert variable efficacy depending on the carrier and the organ target.