RNA Therapeutics, the Pathway to Clinical Application

Jared Beyersdorf, Philip Santangelo

The use of synthetic mRNA to express specific proteins is a highly promising therapeutic and vaccine approach that avoids many safety issues associated with viral or DNA-based systems. Synthetic mRNA is commonly encapsulated with carrier molecules, such as lipid nanoparticles (LNPs) to protect it from degradation *in vivo* and enhance cellular uptake. While LNPs greatly improve trafficking and uptake of synthetic mRNA *in vivo*, many cellular and tissue barriers remain that limit therapeutic application.

One promising target of synthetic mRNA-based therapeutics is the central nervous system (CNS). Synthetic mRNA transfection does not require nuclear entry for protein expression and poses no risk of genomic integration. However, delivery of drugs and other therapeutics to the CNS is limited by the blood-brain barrier (BBB), which prevents LNPs delivered intravenously from transfecting the cells of the CNS directly. To overcome this, we employed focused ultrasound (FUS) to temporarily disrupt the BBB. Focused ultrasound (FUS), currently in Phase I trials, is a safe, noninvasive, and efficient method of disrupting the BBB in distinct locations, allowing for transport of therapeutic agents into the CNS. We have demonstrated in mice that FUS facilitated the movement of intravenously delivered LNPs containing mRNA to the CNS, yielding high levels of protein expression in the brain. While this result is promising, many questions remain to be answered regarding this approach, and obtaining a detailed understanding of the mRNA delivery and protein expression patterns will be crucial to developing effective therapies.

Methods: LNPs were prepared by mixing aqueous mRNA encoding a membrane-bound protein with a V5 epitope tag with organic phase lipids in a microfluidic system. Four animals were anesthetized and their heads placed in the focal point of a custom ultrasound apparatus. Brain images were acquired using a small animal MRI. Magnetic resonance guidance was used to identify and adjust the region of ultrasound application prior to BBB disruption. After focusing, clinical grade albumin microbubbles were injected i.v. and ultrasound was applied to induce stable oscillations. Each of the four mice were then injected with lipid nanoparticles at a volume containing 100 ug of mRNA. The animals were sacrificed 6 hours after injection. Following perfusion, the brains were removed and processed for immunofluorescent staining.

Results: No behavioral changes were observed in the mice after FUS application and LNP delivery. Upon immunofluorescent staining and imaging of the brain tissues, no gross damage to small vessels was observed. High levels of protein expression were observed in cells across many brain regions in both hemispheres. Furthermore, protein expression correlated with levels of LNP uptake across all four animals.

Discussion: These results demonstrate the feasibility of this approach to achieve noninvasive transfection of the CNS using LNPs and synthetic mRNA. Further studies must be conducted to characterize the mechanisms and spatiotemporal patterns of delivery.