CALCIUM PHOSPHATE BIOMATERIALS ENHANCE IMMUNOTHERAPEUTIC mRNA DELIVERY IN MELANOMA

Hannah Martin*, Joshua Choe, Iris Baurceanu, William Murphy. University of Wisconsin-Madison, Madison, WI, USA

Background Messenger ribonucleic acid (mRNA) is a powerful tool for transferring genetic information. Its advantages include potent but transient gene expression without risk of genomic insertion, tailorable immunogenicity to match therapeutic application, and the potential for efficient, scalable manufacturing.1 The recent success of mRNA-based SARS-CoV-2 vaccines has inspired interest in mRNA as a cancer therapy to deliver immunostimulatory molecules and tumor antigens. However, clinical translation is limited by mRNA instability at physiological conditions and inefficient in vivo delivery.2 A reliable, non-toxic, and stabilizing in vivo delivery system for immunotherapeutic mRNA would help to advance mRNA as a viable cancer therapy. Here, we utilized calcium phosphate mineral-coated microparticles (MCMs) as a delivery system for mRNA-lipid complexes (lipoplexes) to transfect melanoma cells.

Methods MCMs were prepared as previously described3 by suspending β-tricalcium phosphate particles in modified simulated body fluid under rotation for 7 days at 37°C, refreshing the media daily. MCMs were then washed in deionized water and freeze dried. Custom-synthesized reporter or therapeutic mRNA constructs were complexed with a lipidic transfecting agent through mixing, then resulting lipoplexes were incubated briefly with MCMs to facilitate electrostatic binding to the porous CaP coating (figure 1a). Loaded MCMs or soluble lipoplexes were added to B16F10 murine melanoma cell culture, and transfection was measured through various assays, including fluorescence microscopy, bioluminescence, and enzyme-linked immunosorbent assays.

Results Scanning electron microscopy was used to verify plate-like, porous coating morphology following MCM fabrication (figure 1b). MCMs enhanced transfection of B16F10 melanoma cells compared to soluble mRNA lipoplex delivery. This was demonstrated with reporter constructs encoding enhanced green fluorescent protein (eGFP, figure 1c) and Gaussia luciferase (G-Luc), as well as with a therapeutic construct encoding interleukin 15 (IL-15), a T cell growth factor. Time-lapse imaging also revealed more rapid transfection with MCMs. A close proximity of cells to MCMs was observed as necessary for transfection.

Conclusions We demonstrated that MCMs efficiently and locally deliver mRNA lipoplexes to melanoma cells and cause elevated levels of protein expression compared to soluble lipoplex delivery. This enhanced delivery profile makes MCMs a potential drug delivery platform for future in vivo tumor studies and clinical translation.

Acknowledgements The research presented was supported under NIH award TL1TR002375. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Thank you to Michael and Mary Sue Shannon for their generous gift towards this project. Artwork in Fig. 1A created on BioRender.com.

REFERENCES

Abstract 1127 Figure 1 Mineral-coated microparticles (MCMs) as an efficient mRNA lipoplex delivery vehicle. (A) Fabrication, loading, and cellular delivery mechanism of MCMs. (B) Scanning electron microscopy image of the surface topography of MCMs (scale bar = 2 um). (C) Representative fluorescence micrograph of B16F10 melanoma cells transfected with MCMs carrying enhanced green fluorescent protein (eGFP) mRNA lipoplexes. eGFP+ cells are shown in green, while nuclei are blue (Hoechst) (scale bar = 200 um).