MRNA VS. SELF-AMPLIFYING MRNA FOR PROTEIN EXPRESSION IN THE RETINA

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Aim: Retinal cell degeneration is a main cause of vision loss. To stimulate retinal cell survival, neurotrophic factors can be administered, for example by delivering messenger RNA (mRNA) encoding these factors. Here, we evaluate the potential and immunogenicity of mRNA and self-amplifying mRNA (SAM) to obtain protein expression in ocular cell types. SAM is expected to produce more protein due to its self-amplifying capacity. Methods: We evaluated the transfection efficiency of mRNA and SAM encoding a green fluorescent reporter protein (eGFP) in a dose titration experiment in ARPE-19 and MIO-M1 cells, two retinal cell types. Their immunogenicity was checked using an interferon-ß (IFN) ELISA kit. Next, we explored two strategies to reduce SAM's immunogenicity: i) B18R (type I IFN decoy receptor) and ii) cellulose-based purification to remove dsRNA contaminants formed during SAM production. Results: Remarkably, mRNA outperformed SAM in the dose titration experiment. This might be explained by the immune response triggered by SAM, which is mediated by type I IFNs, stalls RNA translation and induces RNA degradation. Indeed, IFN-ß production was 50x higher after SAM transfection, compared to mRNA. The impact on RNA translation was confirmed by transfecting type I IFN deficient BHK-21 cells, where SAM outperformed mRNA. Next, two strategies proved to lower SAM's immunogenicity and increase eGFP expression. Co-administration of B18R led to a 4x higher expression, whereas cellulose-based purification of SAM, increased expression 60 and 330x in ARPE and MIO-M1 cells, respectively. Conclusion: Our findings suggest that mRNA and SAM have potential to transfect ARPE-19 and MIO-M1 cells. When using SAM, however, its immunogenicity should be managed to fully exploit its potential.