Research Area Selected - RNA-Based Immune Modulation RNA-based cell fate reprogramming for cancer immunotherapy

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Immunotherapy has revolutionized cancer treatment, but most patients fail to respond due to immune evasion mechanisms. Our group demonstrated that direct reprogramming of tumour cells into type 1 dendritic cells (cDC1s) by intratumoral viral delivery of the transcription factors (TFs) PU.1, IRF8 and BATF3 (PIB) can overcome these challenges and improve antitumor immunity. While clinical translation of this strategy is underway, alternative delivery modalities may offer distinct properties and expand the versatility of direct cell reprogramming approaches. Here, we investigated RNA-based delivery as a complementary, non-viral approach to induce cDC1 reprogramming and explored lipid nanoparticles (LNPs) as a delivery platform. Modified linear RNA (mRNA) and self-amplifying RNA (saRNA) constructs encoding GFP or PIB were synthesised in vitro and evaluated for transgene expression or reprogramming in human dermal fibroblasts (HDFs) and mouse embryonic fibroblasts (MEFs) harbouring the Clec9a-tdTomato DC-specific reporter system. mRNA-mediated expression demonstrated robust but transient transgene expression in HDFs, whereas saRNA showed lower expression with greater persistence over time. RNA-mediated delivery of the PIB factors induced cDC1 reprogramming, with reprogramming efficiency influenced by RNA modality, dose and delivery schedule. Optimization in MEFs showed that lower doses and repeated transfections can enhance RNA-mediated reprogramming efficiency. Additionally, we evaluated LNPs for RNA delivery. LNP-mediated delivery of RNA encoding the PIB factors also supported cDC1 reprogramming, demonstrating the potential of LNPs as a non-viral delivery platform for direct cell reprogramming. Notably, LNP-mediated saRNA delivery induced persistent CD45 expression, peaking at day 3 and remaining detectable between days 2 and 5. Overall, our work provides proof-of-principle for cDC1 reprogramming mediated by RNA across different RNA modalities and delivery approaches. This approach could allow the development of new therapeutics for cancer treatment and expand the available strategies for therapeutic direct cell reprogramming.