A circRNA-based CellREADR platform for cell-type specific effector protein expression

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Background: The therapeutic potential of RNA-based approaches is limited by the lack of cell-type specificity in complex tissues such as the brain. Restricting effector protein expression to defined cell populations is essential to maximize beneficial effects while minimizing adverse side effects. CellREADR (Cell access through RNA sensing by endogenous ADAR) enables cell-specific effector protein expression by coupling translation to the detection of a cell-defining endogenous RNA via A-to-I editing. Here, we adapt the CellREADR system to a circular RNA (circRNA)-based platform containing an antisense sequence targeting TPH2 mRNA to selectively induce brainderived neurotrophic factor (BDNF) expression in serotonergic neurons of the raphe nucleus to promote a stressresilient phenotype. Cell-type specificity is critical, as non-targeted BDNF overexpression has been linked to seizure susceptibility. Method: Circular RNA-based CellREADR constructs containing an antisense sequence to the cell-type-specific marker RNA TPH2 and encoding GFP were generated and compared with their linear mRNA counterparts. READR RNAs were transfected into various cell lines, and target-dependent effector expression was assessed by fluorescence microscopy and flow cytometry. Biodegradable 6-glucosyl-modified block copolymer nanoparticles were synthesized for future systemic READR circRNA delivery. Results: Preliminary in vitro experiments were performed in non-neuronal and neuronal cell lines. We successfully designed and validated several READR sequences targeting TPH2 mRNA and adapted the original DNA-based CellREADR system to mRNA- and circRNA-CellREADR platforms, demonstrating target-dependent effector protein translation. Notably, READR circRNA increased target-dependent effector protein expression while reducing nonspecific expression compared with READR mRNA. Furthermore, we synthesized polymer-based nanoparticles and achieved successful transfection of CellREADR constructs in multiple cell lines. Conclusion: Our results demonstrate the potential of circRNA-based CellREADR as a versatile platform for cellspecific protein expression. Combined with glycosylated polymer nanoparticles to enable systemic delivery across the blood-brain barrier, this approach holds promise for RNA-based interventions targeting defined cell populations in the brain.