Using selectively expressed RNA-loaded nanoparticles to achieve Tph2-dependent BDNF overexpression in serotonergic neurons.

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Background: Chronic stress can lead to hippocampal dysfunction, leading to impaired memory, depression, and further increasing stress susceptibility. Increasing BDNF expression in specific brain regions (namely serotonergic neurons in the raphe nucleus) has been shown to increase hippocampal neurogenesis and elevate depression-like phenotypes, whereas BDNF overexpression in the forebrain or globally can impair working memory and increase anxiety-like phenotypes. Brain region specific gene expression can be achieved through stereotaxic virus administration or breeding of gene-edited animals, but these approaches are restricted in their translational potential. This project will focus on in vitro approaches required to optimise a) the cell-type specific expression of the delivered RNA constructs, b) the use of the polymer or lipid nanoparticle that maximises load and delivery of the RNA into Tph2+ neurons (tryptophan hydroxylase 2, a brain-specific enzyme involved in serotonin synthesis) and c) the transcytosis of the RNA-loaded nanoparticles through brain endothelial cells. Method: Preliminary experiments were performed in vitro by culturing various non-neuronal and neuronal, Tph2- and Tph2+ cell lines. Selectively-expressed RNA (seRNA) encoding GFP (later Bdnf) as a payload protein, the translation of which is dependent on an upstream Tph2 antisense sequence interacting with endogenous Tph2 mRNA, was transfected into cells using conventional liposome reagents or polymer-based nanoparticles. Conjugating seRNA and nanoparticles with Cy3 and Cy5, respectively, allows us to quantify both transfection and translation efficiency. Results: In RN46A-B14 cells (rat raphe nuclei-derived), differentiation and treatment with vitamin D3 increases Tph2 expression levels and the translation of payload protein (GFP) from the seRNA module. Interestingly, GFP expression was even higher in N2a cells, which correlated with a substantially higher Tph2 expression. Conclusion: The current data demonstrates that Tph2 expression positively correlated with GFP translation, but further controls are required to support that endogenous Tph2 mRNA directly interacts with the seRNA to regulate payload translation