CRISPR/Cas9 Based Mechanisms for Controlled Gene Delivery with Applications for Alzheimer's and Pain Management for Veterans

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CRISPR Cas9 is a protein that precisely cuts a sequence complementary to a guide RNA. Recently, a CRISPR/Cas9 based self-cleaving genetic system has been engineered such that DNA can be delivered, expressed for an articulated time, and then destroyed. However, no significant payload has been applied to the design. Thus, the aim of this project was to create a library of self-cleaving vector constructs for transcriptional regulation. Our designs allow for genes to be turned on and off for a user-determined period of time while leaving no trace of the DNA that encoded the transcriptional regulators. The library consists of CRISPR-based self-cleaving plasmids with varying expression times of transcription regulation modulated by mutations in the complimentary gRNA target sequence on the plasmid. Functional domains (tTA, rtTA, LacI, TALE-VP16) were cloned into a mother vector encoding Cas9 and gRNA which target the vector, in order to activate/repress transcription of genes. Then, plasmid functionality was tested using fluorescence microscopy and flow cytometry. A reporter plasmid with TRE-mKATE was used for testing the TALE-VP16 plasmids. Experiments showed a clear pulse of mKATE as the protein is activated by the TALE until the plasmid is cleaved by Cas9 in which mKate is no longer activated. This TALE can be used to target any gene in the human genome. It is anticipated that vital genes in humans such as the SCN9A and BACE1 genes can be activated (or repressed) for a predetermined period of time to increase pain tolerance and slow the onset of Alzheimer’s respectively.