Abstract #1485

Intercellular Zinc Finger Protein Delivery for Cross-corrective Epigenetic Regulation in the CNS

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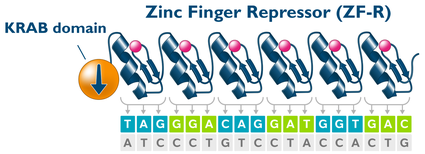
Introduction

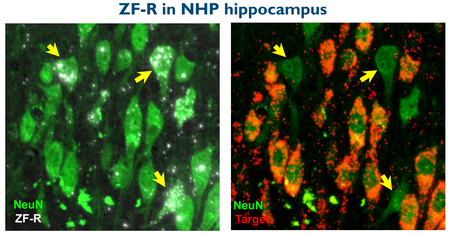
We are developing zinc finger transcriptional regulators (ZF-TRs) to create genomic medicines for the treatment of neurological diseases. The potency, tunability, high specificity and compact size of ZF-TRs has been leveraged to successfully modulate the expression of gene targets involved in an array of neurodegenerative and neurodevelopmental disorders[1,2].

However, widespread brain delivery of therapeutic transgenes remains challenging due to the limited CNS transduction efficiencies of currently available AAV capsids. Cellular secretion of ZF-TRs from AAV-transduced cells and uptake to neighboring cells could provide a new way to achieve broader ZF-TR mediated epigenetic regulation in target CNS cells by cross-correcting non-transduced cells.

Using a secreted luciferase reporter assay with a ZF targeting an endogenous gene fused to the KRAB repressor domain (ZF Repressor, or ZF-R), we identified ZF-R variants compatible with secretion via the secretory pathway in cell lines and primary cells. Furthermore, ZF-R protein was internalized by cells and maintained on-target repression activity highlighting the suitability of ZF-Rs for cross-corrective epigenetic regulation.

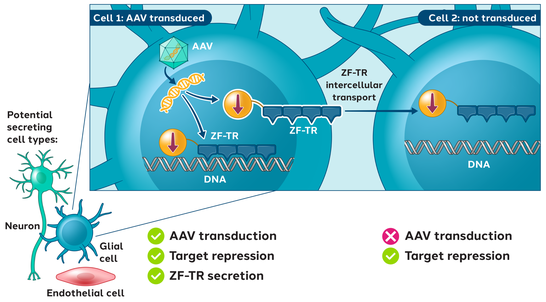


A ZF Repressor mediates potent repression in vivo



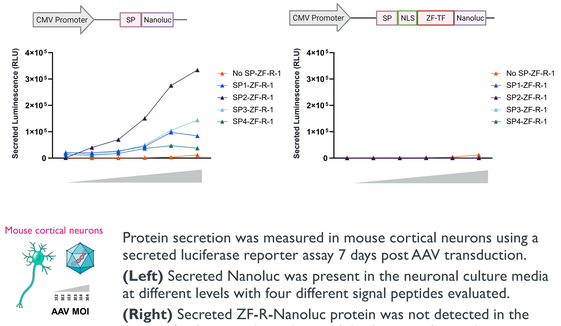
A ZF-R designed to repress an endogenous gene was delivered to a nonhuman primate (NHP) using AAV. IHC staining revealed potent target gene repression in ZF-R+ stained neurons in the NHP hippocampus (yellow arrows). However, not all neurons were transduced with AAV and therefore ZF-R mediated target repression was not observed in all neurons.

ZF-R cross-corrective epigenetic regulation in the CNS



To overcome AAV transduction limitations in the CNS, a new platform technology that enables cross-correction has been developed. ZF-R protein is secreted from AAV transduced cells into the extracellular milieu and taken up into neighboring cells thereby increasing the number of ZF-R containing cells. Different CNS cell types are being explored for cross-corrective epigenetic regulation.

A secreted luciferase reporter assay to measure ZF-R protein secretion in mouse cortical neurons

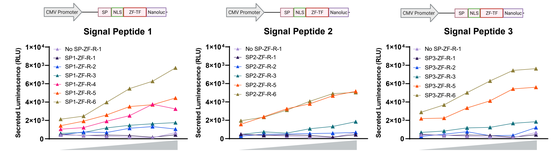


Protein secretion was measured in mouse cortical neurons using a secreted luciferase reporter assay 7 days post AAV transduction.

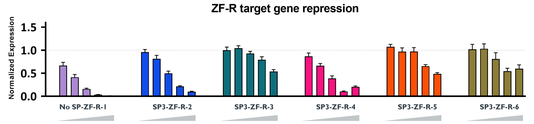
(Left) Secreted Nanoluc was present in the neuronal culture media at different levels with four different signal peptides evaluated.

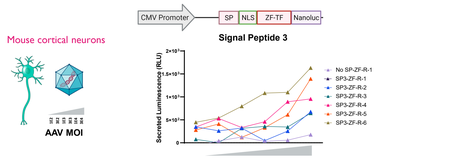
(Right) Secreted ZF-R-Nanoluc protein was not detected in the neuronal culture media with any of the four signal peptides evaluated.

ZF-R variants are secreted from HEK293s, mouse cortical neurons and astrocytes

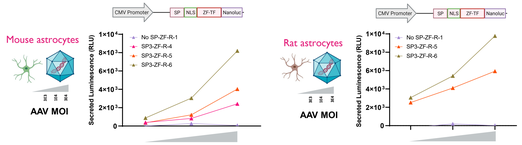


Alternative ZF-R variants were evaluated for secretion in HEK293 cells. 24hr post DNA transfection, the level of secreted protein was measured in the culture media. At the highest dose of transfected plasmid DNA, ZF-R variant SP1-ZF-R-6 was secreted >30-fold more than SP1-ZF-R1 (left panel). Similar levels of secretion improvements were observed with signal peptide 2 (middle panel) and signal peptide 3 (right panel).



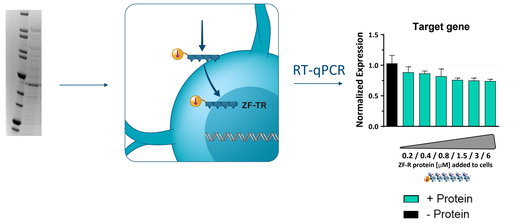


Improved ZF-R secretion variants were evaluated for on-target gene repression activity in mouse cortical neurons and shown to maintain varying levels of repression activity (top panel). Increased levels of secreted protein were also observed in the mouse cortical neuron media compared to a non-secreted (no SP) control ZF-R.



ZF-R secretion was measured from AAV transduced mouse (left panel) and rat (right panel) astrocytes. Secreted ZF-R protein levels were higher than in HEK293 or mouse neurons with >250-fold increase in secreted protein over a non-secreted (no SP) control ZF-R observed in rat astrocytes.

ZF-R protein is cell permeable and regulates gene expression



ZF-R protein was expressed in E.coli, purified and added to mouse neuroblastoma (N2a) cells. After a 6hr incubation, dose dependent repression of the ZF-R target gene was observed (right panel) demonstrating the ability of ZF-R protein to cross the cell membrane and maintain epigenetic regulation activity.

Conclusion

- In order to achieve broader CNS delivery of our ZF Transcriptional Regulators, we identified ZF-R variants capable of protein secretion using the secretory pathway
- The addition of a signal peptide alone was insufficient for ZF Repressor (ZF-R) secretion through the constitutive secretory pathway in mouse cortical neurons
- Enhanced protein secretion of ZF-Rs was observed in HEK293s, mouse cortical neurons and astrocytes with a >250-fold increase in secretion observed
- ZF-R variants maintained on-target gene repression activity in mouse cortical neurons
- ZF-R protein was shown to be cell permeable, maintain on-target repression activity and demonstrated ZF-R protein is compatible with a cross-corrective epigenetic regulation approach
- Future work using assays to measure cellular secretion and uptake in relevant cell types will be investigated to identity ZF-Rs capable of cross-corrective epigenetic regulation in vitro and in vivo
- Intercellular delivery could significantly increase the number of CNS cells exposed to ZF Transcriptional Regulators, thus increasing the potential therapeutic benefit for a range of CNS disorders.

References

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2. Zeitler B, Froelich S, Marlen K, et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease. *Nat Med.* 2019; 25(7):1131-1142

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