Abstract #1057

Gene Activation Mediated by Zinc Finger Transcriptional Regulators as a Therapeutic Approach for CNS Disorders

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Introduction

Neurodevelopmental disorders are impairments of the development of the brain and/or central nervous system (CNS) that affect emotion, learning ability, self-control, and memory. The development of the nervous system is tightly regulated and influenced by both genetic and environmental factors. Some neurodevelopmental disorders are considered multifactorial syndromes which have many causes that converge to a more specific neurodevelopmental manifestation in the form of genetic haploinsufficiency.

We developed Zinc Finger Activators (ZF-As) to upregulate the expression of a target gene as a potential therapeutic for neurodevelopmental disorders caused by haploinsufficiency. ZF Activators are obtained by tethering Zinc Finger Proteins (ZFPs) to a trans-activation domain (Fig. 1).

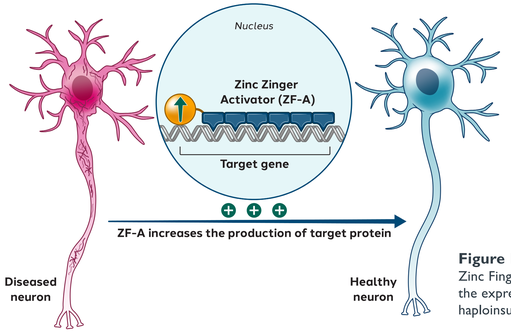
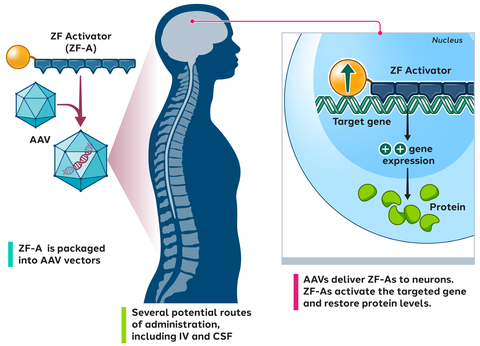


Figure 1: Schematic representation of Zinc Finger Activators (ZF-As) upregulating the expression of a target gene to rescue haploinsufficiency-associated phenotypes.

Zinc Finger Activator (ZF-A) Platform

Figure 2: Schematic representation of the ZF-A platform, using AAV as the delivery vector, upregulating the expression of a target gene.

Neurodevelopmental disorders of haploinsufficiency present promising therapeutic targets.



Method: Zinc Finger Activator In Vitro On-Target and Specificity Assessment

We aim to upregulate target gene protein expression using our Zinc Finger protein technology and AAV delivery platform (Figure 2). ZF-As can enable modulation of target protein expression and alleviate the phenotypes associated with haploinsufficiency. We examined the ability of ZF-As to upregulate target gene expression and associated specificity for their target gene (Figure 3).

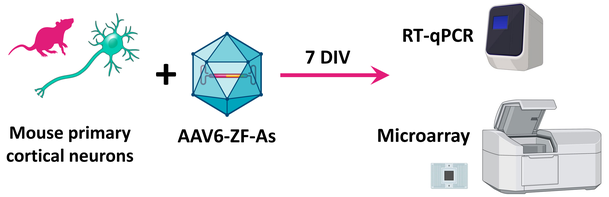


Figure 3: Schematic representation of the workflow examining the efficacy of ZF-As in vitro.

Mouse cortical neurons were transduced with AAV6 harboring ZF-As. 7 days post-transduction, the cultures were harvested and examined for on-target upregulation via qPCR. Specificity for the target gene was assayed using microarray analysis. Figure created with assistance from BioRender.com.

Results: Zinc Finger Activators Demonstrate up to 4-Fold Target Gene Upregulation and High Specificity In Vitro

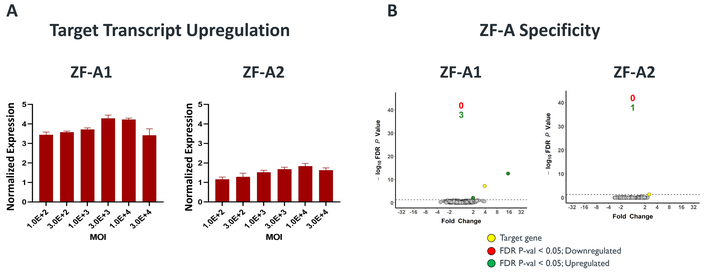


Figure 4: ZF-As demonstrate varied on-target activation and high specificity in vitro.

(A) Primary mouse cortical cultures were transduced at 6 different MOIs. ZF-A1 and ZF-A2 upregulated target gene expression up to 4- and 1.5-fold respectively.

(B) Microarray analysis was performed to assess the specificity of each ZF-A construct in primary mouse cortical cultures 7 days post-transduction, using 3E3 MOI. ZF-A1 and ZF-A2 demonstrate minimal differentially expressed genes (DEGs) indicating high target gene specificity.

Results: ZF-As Demonstrate Target Gene Upregulation In Vivo

We further examined whether ZF-As can upregulate target mRNA and protein expression in vivo (Fig. 5). In this study, AAV ZF-A1 was delivered to 6-week-old C57BL/6 wildtype mice via intravenous injection. Four weeks later, target mRNA and protein levels were assessed.

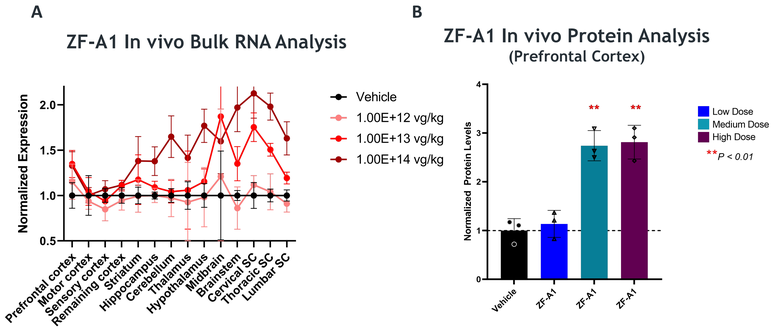


Figure 5: ZF-A1 upregulates target mRNA and protein expression in vivo.

(A) Bulk RT-qPCR analysis demonstrates in vivo target engagement and upregulation at various doses.

(B) Western blot analysis of prefrontal cortex demonstrated up to 3-fold increase in the gene target protein following ZF-A1 administration.

Conclusion

- ZF Activators can mediate target gene upregulation in vitro and in vivo across a wide range of doses.
- These data demonstrate the therapeutic potential for ZF-As to be developed for neurodevelopmental disorders caused by haploinsufficiency.

Acknowledgments

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