Shank3 Gene Activation Mediated by Zinc Finger Activators (ZF-As) as a Therapeutic Approach for Phelan-McDermid Syndrome

Poster P415

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Introduction

Phelan-McDermid syndrome (PMS) is a rare genetic condition characterized by clinical features with varying severity, including intellectual disability, absent or delayed speech, and autism spectrum disorders (ASD)[1].

PMS is caused by a deletion or structural change in chromosome 22 or a pathogenic variant of the *SHANK3* gene. *SHANK3* encodes the synaptic protein SHANK3, localized at excitatory synapses. SHANK3 plays a major role in organizing scaffolding proteins, which are crucial for proper synapse formation and dendritic spine maturation. Mutations or loss of a *SHANK3* allele due to copy number variations can lead to *SHANK3* haploinsufficiency, causing synaptic and circuitry deficiencies associated with PMS[1,2].

We designed Zinc Finger Activators (ZF Activators, or ZF-As) targeting the mouse *Shank3* gene and assessed *Shank3* mRNA and SHANK3 protein levels in cultured mouse cortical neurons. ZF Activators are obtained by tethering Zinc Finger Proteins (ZFPs) to a trans-activation domain (Fig. 1).

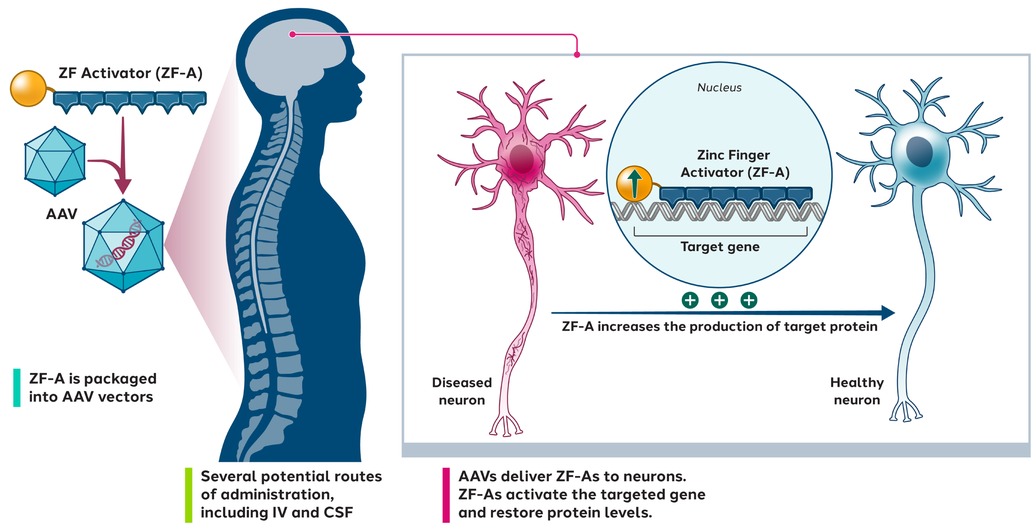


Figure 1: Schematic representation of the ZF-A platform, using AAV as the delivery vector, upregulating expression of *Shank3* in neurons to rescue haploinsufficiency phenotypes.

Study design

Candidate Shank3 ZF-As manufactured for AAV viral delivery were tested in vitro and in vivo. Downstream readouts included RNA and protein on-target activity, as well as specificity analyses by examining off-target profiles using a microarray platform (Fig. 2).

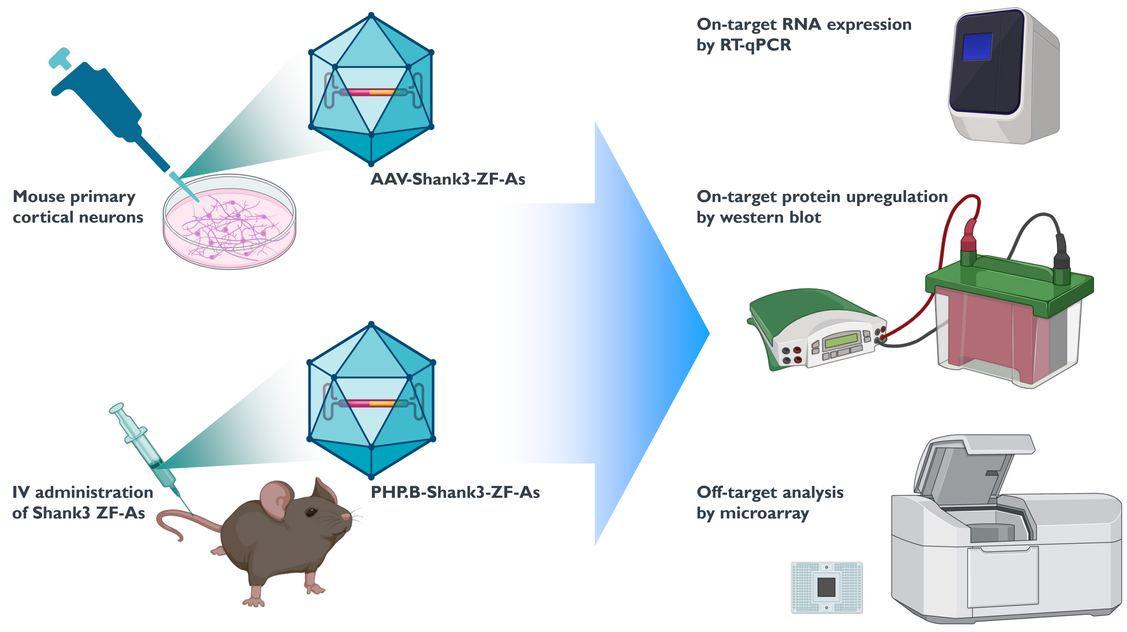


Figure 2: Schematic representation of the workflow examining the efficacy of ZF-As in vitro and in vivo. Mouse cortical neurons were transduced with AAV harboring Shank3 ZF-As.

- For in vitro studies, cultured neurons were harvested 7 days post-transduction to assess RNA and protein on-target activity and off-target analysis by microarray.
- For in vivo studies, brains were collected from adult mice 4 weeks after treatment with PHP.B encoding Shank3 ZF-As via intravenous (IV) tail vein injection.

Figures created using BioRender.com.

Results

1 SHANK3 ZF-As are highly specific and achieve up to 4-fold upregulation in cortical neurons in vitro

We designed and assembled SHANK3 ZF-As targeting DNA regions within the *Shank3* locus. Assembled ZF-As were screened for on-target activity in Neuro2A cells, leading to the identification of several ZF-As able to upregulate *Shank3* (data not shown). A subset of ZF-As with a range of on-target activity were manufactured in AAV and tested for on-target and specificity in cultured cortical neurons dissociated from wildtype mice (Fig. 3).

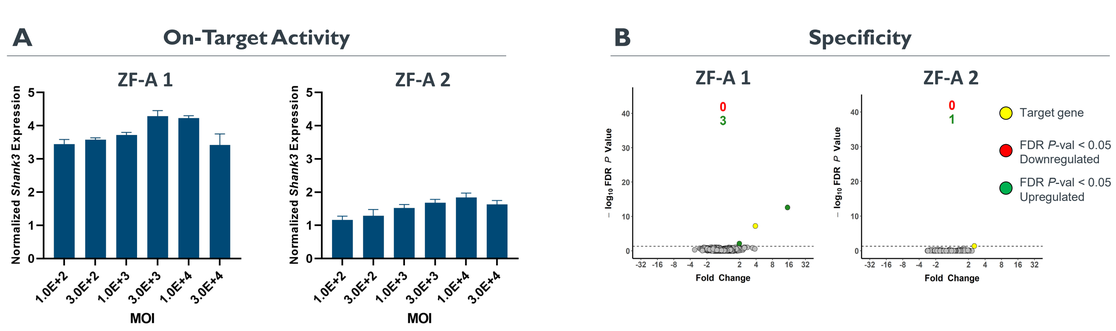


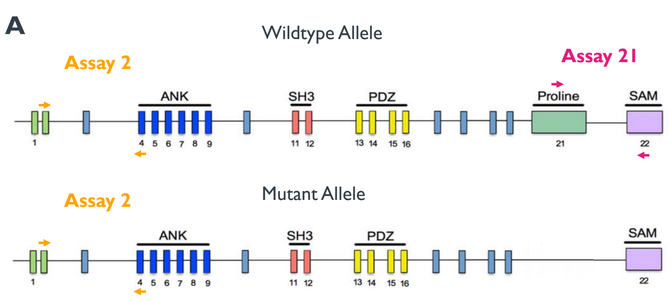
Figure 3: ZF-As demonstrate varied on-target activation and high specificity in vitro.

(A) Primary mouse cortical cultures were transduced at 6 different doses (MOI = multiplicity of infection). ZF-A 1 and ZF-A 2 upregulated target gene expression up to 4- and 1.5-fold, respectively. Error bars indicate standard deviation (SD).

(B) Microarray analysis was performed to assess the specificity of each ZF-A construct in primary mouse cortical cultures 7 days post-transduction. ZF-A 1 and ZF-A 2 demonstrate minimal differentially expressed genes (DEGs) indicating high target gene specificity.

2 ZF-As activate *Shank3* expression in cultured cortical neurons from a mouse model of Phelan-McDermid Syndrome

Shank3$^{\Delta C21}$ mice lack exon 21, causing the production of a truncated SHANK3 protein due to a missing C-terminal region. Mice heterozygous for this allele (Shank3$^{+/\Delta C21}$ or HET) exhibit behavioral phenotypes indicative of ASD. Thus, these mice are used to study ASD, particularly Phelan-McDermid Syndrome[3].



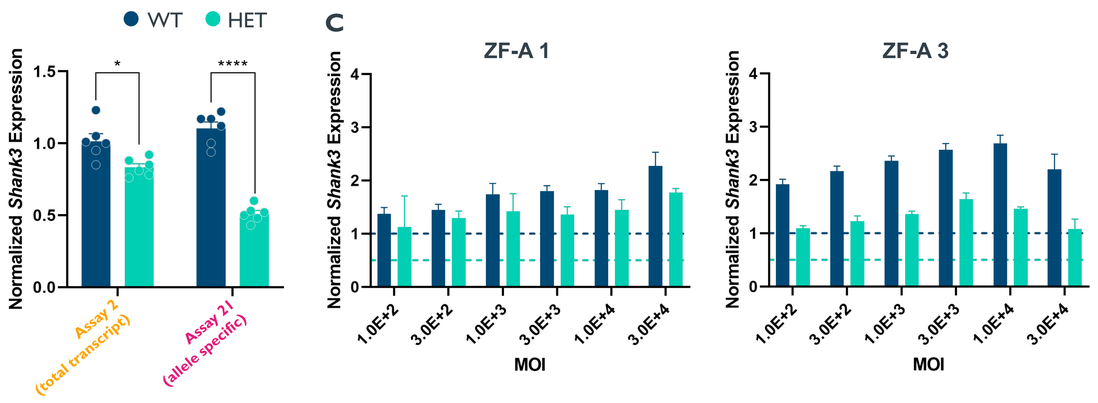


Figure 4: SHANK3 ZF-A upregulates *Shank3* expression in cortical neurons from PMS mouse model.

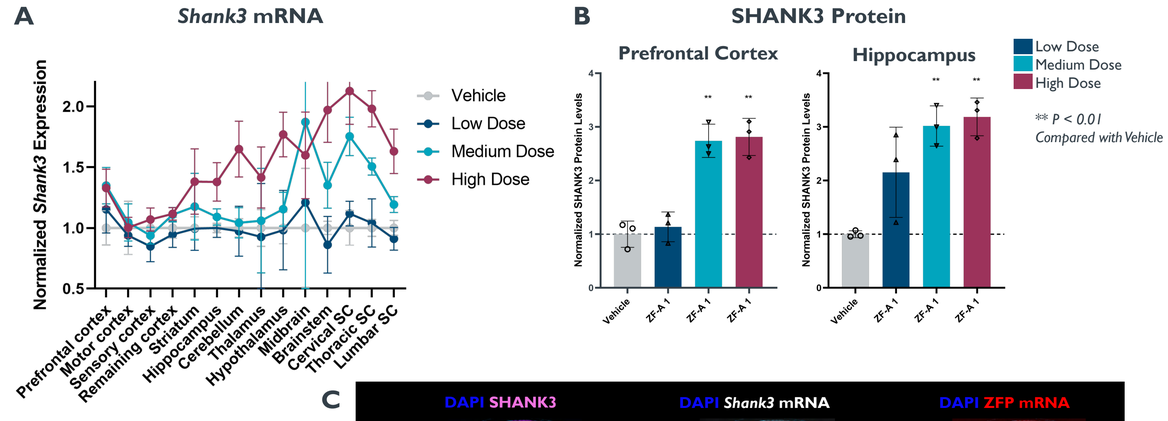
(A) Schematic diagram of the *Shank3* locus with regions encoding functional domains and motifs in wildtype and those lacking in the Shank3$^{\Delta C21}$ mutant allele. Arrows label RT-qPCR probe sets (Assay 2 and Assay 21) used for allele-specific amplification.

(B) Bulk RT-qPCR analysis demonstrates downregulation of the mutant allele in cortical neurons dissociated from the Shank3$^{\Delta C21}$ mouse brain. Error bars indicate standard error of the mean (SEM); * $P < 0.05$; **** $P < 0.0001$.

(C) Bulk RT-qPCR analysis of cortical neurons after transduction of AAV-Shank3 ZF-A shows dose-dependent upregulation of *Shank3* expression in both WT and HET cortical neurons. Error bars indicate standard deviation (SD).

3 ZF-As demonstrate up to 3-fold SHANK3 upregulation in vivo

We examined whether SHANK3 ZF-As can upregulate target mRNA and protein expression in vivo. In this study, PHP.B vectors encoding Shank3 ZF-A constructs were delivered intravenously in a 6-week-old C57BL/6J wildtype mouse. Four weeks later, *Shank3* mRNA and SHANK3 protein levels were assessed from bulk and sectioned tissues. Upregulation of *Shank3* mRNA was detected in various regions of the brain. We notably observed a significant increase in SHANK3 protein levels in ASD-relevant forebrain regions, the prefrontal cortex and hippocampus, confirming ZF-A target engagement.



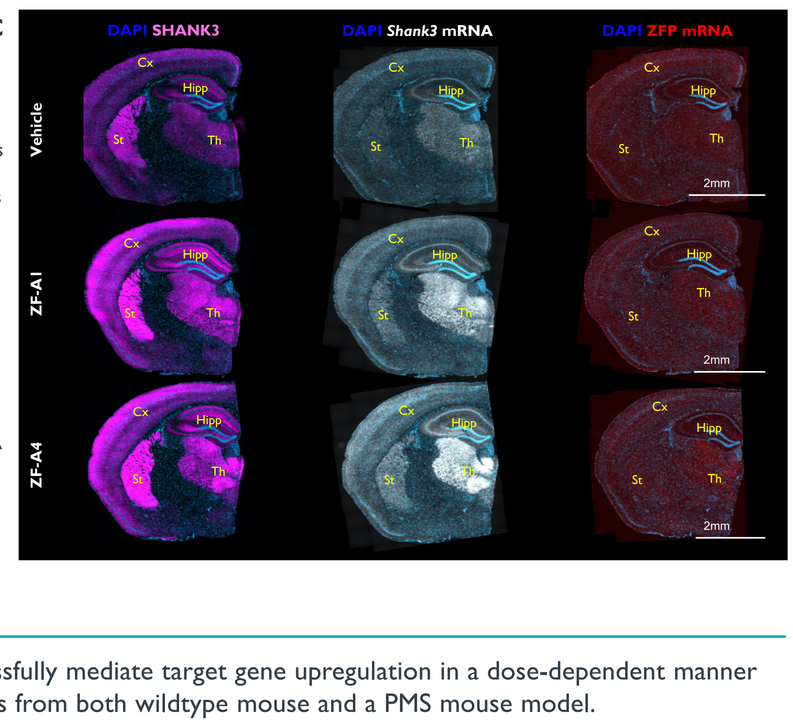


Figure 5: ZF-A 1 upregulates target mRNA and protein expression in vivo.

(A) Bulk RT-qPCR analysis demonstrates in vivo target engagement and upregulation at various doses. Error bars indicate standard deviation (SD).

(B) Western blot analysis of protein lysates from the prefrontal cortex and hippocampus demonstrated a dose-dependent and up to 3-fold increase in SHANK3 protein following Shank3 ZF-A1 administration. Error bars indicate standard deviation (SD).

(C) IHC/ISH analysis demonstrated ZFP-mediated increase in *Shank3* mRNA and protein signal in various regions, including the cortex, hippocampus, striatum, and thalamus.

Conclusion

- SHANK3 ZF-As can successfully mediate target gene upregulation in a dose-dependent manner in cultured cortical neurons from both wildtype mouse and a PMS mouse model.
- SHANK3 ZF-As successfully achieved target engagement, particularly in brain regions implicated in Phelan-McDermid Syndrome or Autism Spectrum Disorders, suggesting therapeutic potential for SHANK3 ZF-As.
- These data support further development and investigation of ZF-As as a therapeutic option for neurodevelopmental disorders caused by genetic haploinsufficiency.

Acknowledgments

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References

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