Cell-type Specific Reduction of Prion Expression in Neurons and Astrocytes using Engineered Zinc Finger Transcriptional Regulators

Abstract #1561

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Introduction

- Prion disease is a fatal neurodegenerative disorder caused by misfolding and aggregation of the prion protein, PrP, encoded by the PRNP gene. Cellular PrP is ubiquitously expressed throughout the body and PRNP transcripts are abundant in both neurons and glial cells.
- There are currently no approved or clinical-stage disease-modifying therapies for the prevention or treatment of prion disease. We are developing a potential single administration therapeutic approach using Zinc Finger Repressors (ZF-Rs) to lower the expression of brain PrP.
- Several lines of evidence from prion-infected mouse models suggest that neuronal PrP expression is necessary and sufficient for neurotoxicity and disease progression[1-3]. We have investigated the cell-type specificity of different promotors paired with a *Prnp* ZF-R at the tissue and single-cell level.

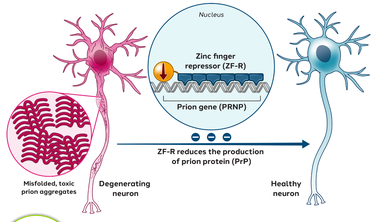

Figure 1. ZF-Rs reduce prion expression and prevent neurodegeneration in prion disease.

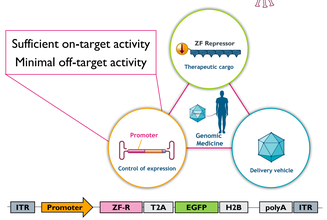

Figure 2. Strategy to evaluate cell-type specific ZF-R repression of prion expression.

Experimental design

- We previously identified highly potent ZF-Rs that reduced >90% of *Prnp* expression with no detectable off-target activity in primary mouse cortical neurons (MCN).
- We expressed a *Prnp*-targeted ZF-R using one of three promoters with known expression profiles: hSYN1 (neuron), GfaABC1D (astrocyte), or CMV (ubiquitous).
- We delivered these constructs using adeno-associated virus (AAV) to wildtype adult mice to determine how much *Prnp* reduction could be achieved when expressing the ZF-R in different cell populations. Analyses were done both at the bulk and single-cell level to evaluate the cell-type specificity of each promoter.
- We also tested these promoters in vitro using human iPSC-derived astrocytes and primary mouse neurons and astrocyte to assess *Prnp* and PrP reduction.

Results

ZF-R reduced >50% of *Prnp* & PrP expression in mouse brain

- Adult wildtype mice with AAV-ZF-R treatments showed a significant reduction of *Prnp* mRNA expression at the bulk level in brain and spinal cord (hSYN1 ≥ CMV > GfaABC1D) via RT-qPCR analysis, depending on the region analyzed.
- The ZF-R driven by the hSYN1 promoter achieved specific expression and *Prnp* repression in the brain and spinal cord; whereas CMV and GfaABC1D were expressed and active in peripheral tissues.
- The hSYN1-ZF-R reduced bulk PrP protein levels by 57% in the brain.

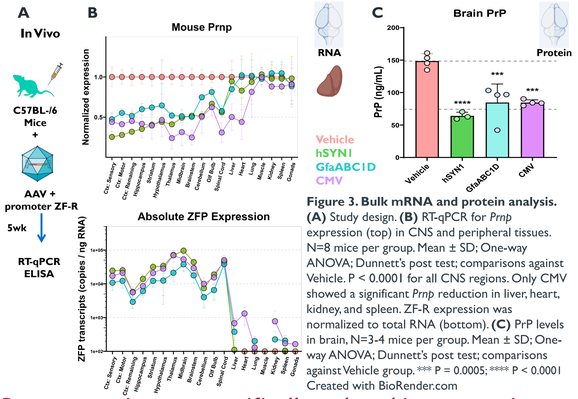

Figure 3. Bulk mRNA and protein analysis. **(A)** Study design. **(B)** RT-qPCR for *Prnp* expression (top) in CNS and peripheral tissues. N=8 mice per group. Mean ± SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle. P < 0.0001 for all CNS regions. Only CMV showed a significant *Prnp* reduction in liver, heart, kidney, and spleen. ZF-R expression was normalized to total RNA (bottom). **(C)** PrP levels in brain, N=3-4 mice per group. Mean ± SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group. *** P = 0.0005; **** P < 0.0001 Created with BioRender.com

Prnp expression was specifically reduced in neurons in hSYN1-ZF-R treated mouse cortex

- Single nucleus 10x transcriptomic analysis of mouse cortex revealed promoter-dependent specificity of *Prnp* repression for neurons and glia.
- *Prnp* reduction was observed for all groups in both excitatory and inhibitory neurons, with the hSYN1 resulting in the most potent and selective effect.
- The GfaABC1D group displayed no significant reduction of *Prnp* in glial cells.
- The CMV group had significant *Prnp* reduction in all four cell populations.

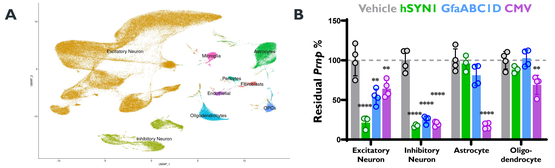

Figure 4. Single-cell transcriptomic analysis. **(A)** Uniform Manifold Approximation and Projection (UMAP) plot showing single-cell gene expression profiles. Clustering and brain cell type assignment based on selective marker genes. Prion levels were assessed for cell populations where individual total cell counts were >=1000 per treatment. **(B)** Normalized residual *Prnp* expression for different cell types in cortex. Error bars are Mean ± SD . One-Way ANOVA with Dunnett's comparisons; ** P < 0.01, **** P < 0.0001.

hSYN1-driven ZF-R repressed *Prnp* expression efficiently and specifically in mouse neurons in the brain

- To assess *Prnp* repression at the single-cell level *in vivo*, we used multiplexed RNAscope (ZF-R, *Prnp*) with immunohistochemistry (GFP, NeuN, S100β).
- For all promoters, a strong negative correlation between ZF-R and *Prnp* expression was observed throughout the brain.
- In all brain regions examined, the hSYN1 promoter resulted in neuron-specific expression, the CMV promoter drove heterogenous expression primarily in neurons and astrocytes, and the GfaABC1D promoter showed minimal expression in astrocytes and weak expression in neurons.

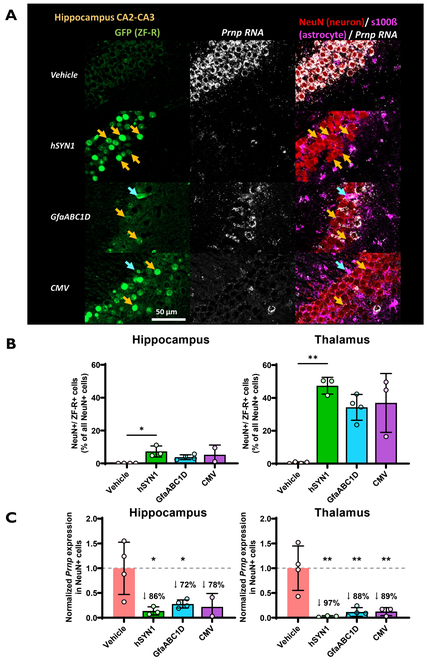

Figure 5. Single-cell analysis via RNAscope. **(A)** Representative images from mouse hippocampal CA2 region. Blue and yellow arrows are astrocytes and neuron transduced with ZF-R, respectively. **(B)** Quantification of % ZF-R transduction in NeuN+ cells per animal. Mean ± SD; Kruskal-Wallis test; comparisons against Vehicle group; * P = 0.012, ** P = 0.009. **(C)** Quantification of vehicle-normalized *Prnp* expression in NeuN+ cells. Mean ± SD; One-Way ANOVA with Dunnett's comparisons; * P < 0.05, ** P < 0.005.

hSYN1 drove neuron-selective repression of mouse and human prion *in vitro*

- ZF-R expression and prion repression was assessed using RT-qPCR and IHC in primary mouse neurons and astrocytes, and human iPSC-derived astrocytes.
- ZF-R expression and prion repression varied depending on the promoter and cell type.
- Prion reduction in astrocytes was detected with CMV and GfaABC1D but not hSYN1.

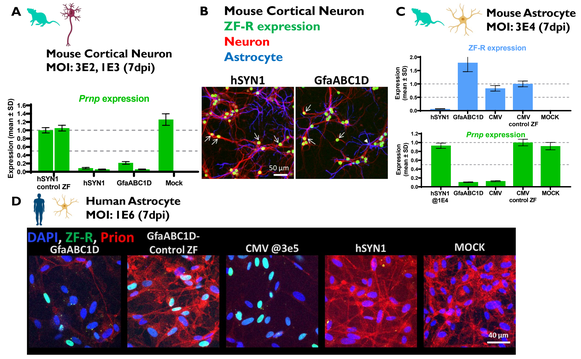

Figure 6. Evaluating expression specificity and prion repression in vitro. **(A)** *Prnp* expression after AAV transduction in MCNs. *Prnp* levels were normalized to the mean of *Atp5b* and *Eif4a2*. **(B)** Confocal image of AAV-ZF-R-GFP-H2B transduced MCN stained with anti-MAP2 for neurons and anti-GFAP for astrocytes. Transduced neurons (white arrows) and astrocytes (white arrowhead). **(C)** AAV transduced mouse astrocytes showed expression of ZF-R and potent *Prnp* repression. **(D)** Representative confocal images demonstrated expression of ZF-R (green) and prion protein (anti-POM2, red) in of human iGABA-derived astrocytes transduced with AAV-ZF-R at 7 days post infection (dpi). Created with BioRender.com

Conclusion

- For all promoters, a strong negative correlation between ZF-R treatment and *Prnp* expression was observed at the single-cell level throughout the brain.
- Neuron-specific expression was observed for the hSYN1 promoter in all brain regions examined. For the CMV group, heterogenous expression was observed, primarily in neurons and astrocytes. In contrast to previous reports, the GfaABC1D promoter did not appear to drive selective expression in astrocytes, but rather in neurons, albeit weaker than hSYN1.
- The potency and selectivity of neuron-specific ZF-R expression and PrP repression support the further development of AAV ZF-Rs for the potential treatment of prion disease.

References

1. Brandner S, et al. "Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system". Proc Natl Acad Sci U S A. 1996;93(23):13148–51
2. Mallucci, Giovanna et al. "Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis." Science (New York, N.Y.) vol. 302,5646 (2003): 871-4.
3. Lakkaraju, Asvin K K et al. "Glial activation in prion diseases is selectively triggered by neuronal PrPSc." Brain pathology (Zurich, Switzerland) vol. 32,5 (2022): e13056.

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