Selective repression of C9ORF72 repeat expansion-containing sense and antisense transcripts for the treatment of ALS

Abstract#577

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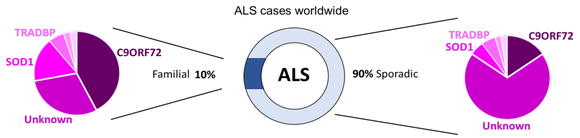
Abstract

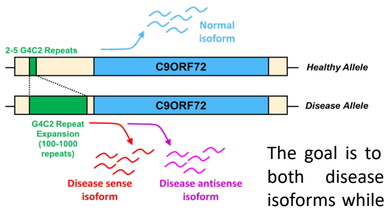
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by the loss of motor neurons in the CNS, leading to paralysis and early death. The most frequent genetic cause of ALS is the expansion of hexanucleotide GGGGCC (G4C2•G2C4) repeats in the first intron of C9ORF72 gene. Analysis of patient autopsy tissue shows that G4C2•G2C4 repeats undergo bidirectional transcription generating sense and antisense expansion containing RNA and RNA foci as well as repeat-derived dipeptide translation products, suggesting a pathological, gain-of-function mechanism. To decrease the levels of expansion–containing transcripts in cells, while maintaining expression of healthy C9ORF72 mRNA levels, we designed a library of engineered transcription factors comprised of a zinc finger protein (ZFP) specifically targeting the G4C2 repeat region fused to a DNA-binding repressor protein (KRAB). Using patient-derived fibroblasts, we were able to identify ZFP-TFs that selectively repress >90% of both sense and antisense G4C2 containing transcripts over a wide dose range while preserving the expression of >50% of C9orf72 mRNA levels. Expression of other G4C2-containing genes was minimally affected, and we identified several ZFP-TFs with minimal to no off-target activity. To confirm the effect of these ZFP-TFs in a disease-relevant cell model, we generated motor neurons from patient-derived iPSCs carrying ~1200 G4C2 repeats. Similar to the effect observed in fibroblasts, the selected ZFP-TFs, packaged in AAV also displayed >90% reduction of both sense and antisense G4C2 containing transcripts over a wide dose range while preserving the expression of >50% of C9orf72 mRNA levels. Minimal to no modulation of other genes was observed by transcriptomics analysis. These findings illustrate the potential use of ZFP-TFs for the treatment of familial ALS.

Background

Expansion of G4C2 repeat in C9ORF72 gene is responsible for 30-40% of ALS familial cases worldwide

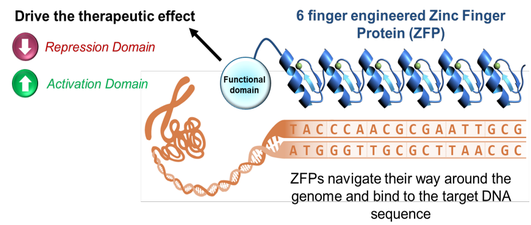


Accumulation of disease sense and antisense isoforms leads to the loss of motor neurons and the manifestation of the disease



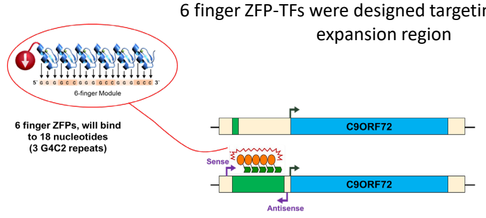
The goal is to repress the expression of both disease sense and antisense isoforms while preserving the expression of the normal isoform as a therapeutic approach for ALS

1. Zinc fingers can be linked to a functional domain (ZFP-TF) to generate targeted therapeutics



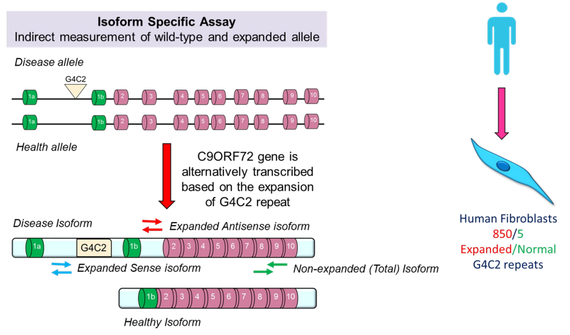
A zinc finger is a naturally-occurring protein structure in many transcription factors and nuclear proteins found in the human body

2. ZFP-TFs were assembled targeting different frames of G4C2 sequence on the top or bottom strands

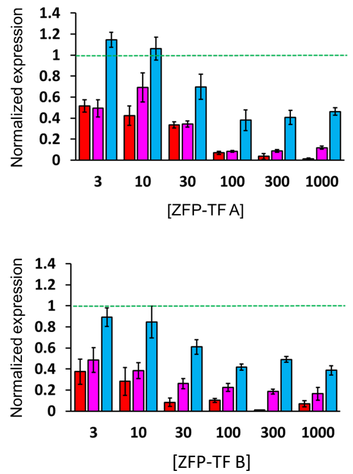


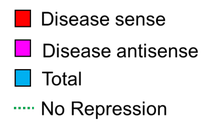
6 finger ZFP-TFs were designed targeting the repeat expansion region

3. Isoform Specific Assay was used to detect isoform specific repression by using qPCR in human primary fibroblasts



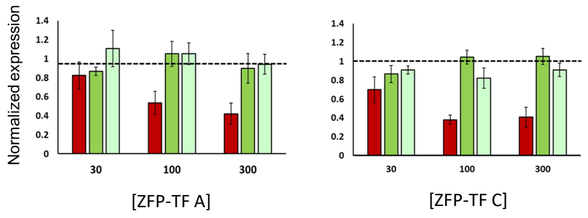
4. ZFP-TFs are capable of maintaining isoform-selectivity over a wide dose range





Multiple ZFP-TFs were identified capable of selectively repressing the expression of both disease sense and antisense isoforms over a wide dose range while preserving the expression of normal isoform in patient derived fibroblast cells

5. The repression of total isoform is minimally affected in healthy cell lines with different G4C2 repeat numbers



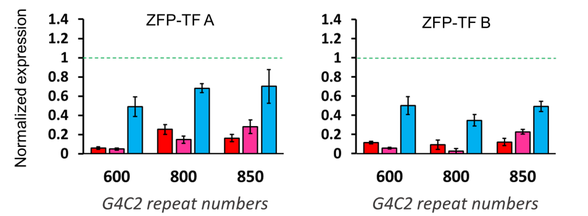
Disease cell line (850/5) - (# of G4C2 repeats for each allele)

Healthy cell line (8/5)

Healthy cell line (20/5)

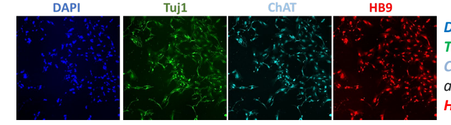
- The repression of the total isoform by selective ZFP-TFs was evaluated in two different healthy lines with different G4C2 repeat length
- The repression of total isoform in disease line (850/5) is a consequence of the repression of the disease isoforms because the qPCR assay for total isoform recognizes both disease isoforms and normal (healthy) isoform (the qPCR assay for total isoform targets exons 8 and 9 as shown figure 3)
- The expression of the normal isoform is minimally affected by the selective ZFPs

6. ZFP-TFs are able to repress the expression of disease isoforms in multiple patient derived fibroblast lines with different repeat numbers



300ng of each ZFP-TF was transfected into each patient derived fibroblast cell line containing different G4C2 repeat numbers on their disease allele

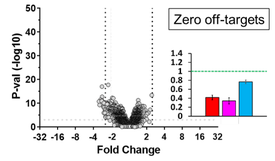
7. ZFP-TFs are able to repress the disease isoforms in patient derived motor neurons with minimal off-target gene regulation detected



DAPI: *Nucleus*
Tuj1 *(class III beta-tubulin)*
ChAT, choline acetyltransferase
HB9*, homeobox gene*

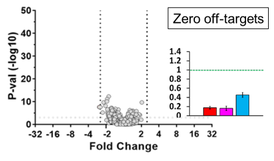
- C9-iMNs were transduced with ZFP-AAV6 at MOI of 30k to evaluate off-target effects of ZFP-TFs in patient derived motor neurons
- Cells were transduced after a week of differentiation and incubated with ZFP-TFs for another week after that
- Inset figure: The on-target qPCR analysis

Global transcriptomic analysis



ZFP-TF A

Global transcriptomic analysis



ZFP-TF B

Fold change +/-2
P-Val: 0.001

Summary and Conclusions

- Multiple ZFP-TFs were identified capable of selectively repressing the expression of both disease sense and antisense isoforms over a wide dose range while preserving the expression of normal isoform in patient derived fibroblast cells and motor neurons
- Global transcriptomic analysis identified ZFP-TFs with no detectable off-target gene regulation
- Studies ongoing to evaluate ZFP-TFs in C9ORF72 mouse BAC models

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