UBE3A Gene Activation Mediated by Zinc Finger Activators (ZFAs) as a Therapeutic Approach for Angelman Syndrome

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Sangamo THERAPEUTICS

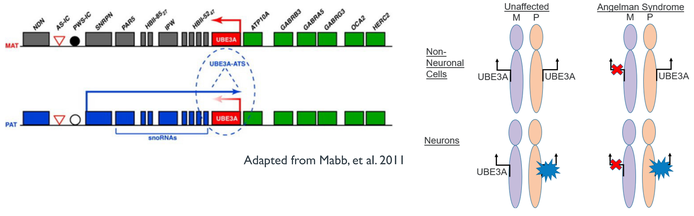
Poster #1121

Introduction

- Angelman syndrome (AS) is a complex and severe neurodevelopmental disorder that primarily affects the central nervous system (CNS) and is often diagnosed in early childhood.
- Hallmark symptoms include intellectual disability, severe speech impairment, developmental delays, movement issues and seizures.
- The genetic cause of AS involves the pathological loss of expression from a single allele of the *UBE3A* gene, coupled with the typical epigenetic silencing of the second *UBE3A* allele in neurons.
- Our goal was to identify a one-time, neuron-specific, therapeutic approach capable of increasing expression of *UBE3A* from the silenced allele and ultimately restoring physiological levels of UBE3A in neurons.
- We show that AAV packaged ZFAs increase both *UBE3A* transcript and UBE3A protein in patient-derived neurons and that surrogate ZFAs increase *Ube3a* transcript in primary neurons sourced from a disease mouse model of AS.

Tissue-specific imprinting: in neurons, the paternal *UBE3A* allele is epigenetically silenced, whereas it is readily transcribed in other cell types.

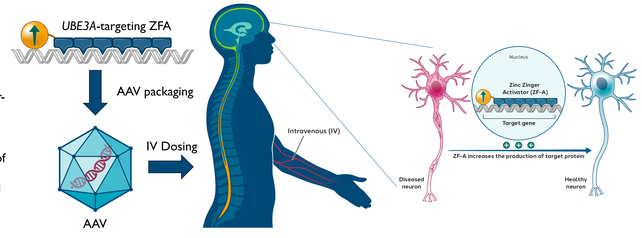
- The LNCAT is a Large Noncoding Antisense Transcript expressed in the paternal allele of neurons that contains the *UBE3A*-ATS on its distal end (full gene: SNHG14).
- In neurons, it is thought that overlap of the LNCAT with the *UBE3A* gene body blocks full length *UBE3A* transcript from being formed - leading to loss of UBE3A protein expression from the paternal allele and creating an effective knockout of UBE3A expression in neurons.



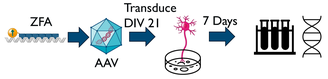
Adapted from Mabb, et al. 2011

Leveraging ZFAs for the potential treatment of Angelman syndrome

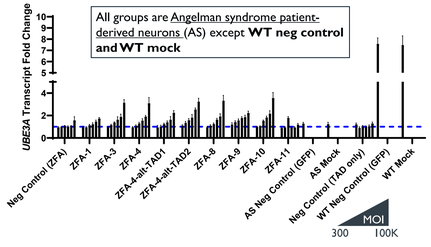
Angelman syndrome is a disease of neurons throughout the brain. Utilizing a blood brain barrier-penetrant AAV capsid would be optimal for systemic delivery of our *UBE3A*-targeting ZFAs and brain-wide biodistribution to affected neurons.



ZFAs increase expression of *UBE3A* in a patient-derived neuron line

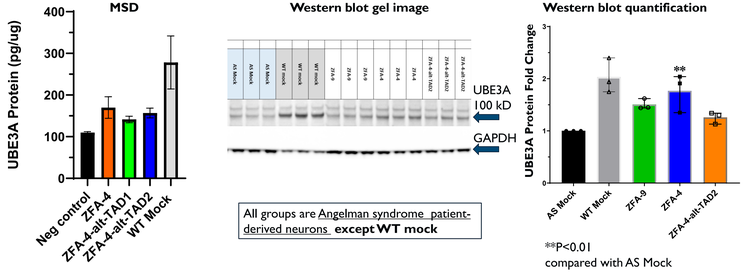


- A large-scale screen of *UBE3A*-targeting ZFAs was performed in a human cell line via nucleofection (data not shown) and the best performing ZFAs were selected based upon their on-target activity profile.
- To determine if these ZFAs could activate *UBE3A* from the healthy, silenced paternal allele in neurons, patient-derived neurons (indicated as "AS") containing a large deletion in the maternal allele of *UBE3A* were cultured until DIV 21 (when cell maturity was high and *UBE3A* expression levels stable).
- Neurons were transduced with AAV vector containing ZFAs and a human synapsin promoter at six doses and then harvested for RT-qPCR analysis at DIV 29.
- Several ZFA's showed increases in *UBE3A* expression in a dose-dependent manner. ZFA-4 was designed and tested with a variable configuration, encompassing 3 separate transactivation domains (TADs).



Select ZFAs increase expression of human UBE3A protein

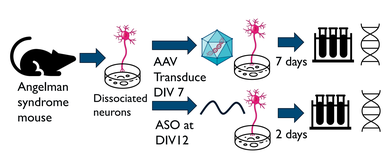
Using similar culture conditions as above, we utilized two orthogonal methods for determining UBE3A protein levels in neurons; MSD and Western blot. All experiments were performed in Angelman syndrome patient-derived neurons, unless noted as WT. 3 different TADs were engineered into ZFA-4 to better understand the modularity of ZFAs.



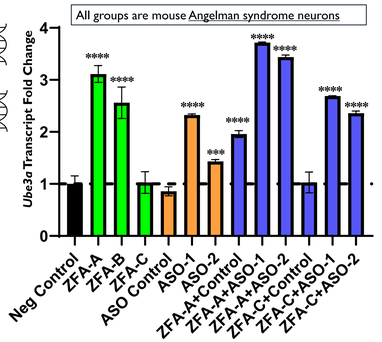
All groups are Angelman syndrome patient-derived neurons except WT mock

**P<0.01
compared with AS Mock

Select ZFAs increase expression of mouse *Ube3a* in disease neurons



- Due to lack of conservation between human and mouse genetics, we generated surrogate mouse ZFA reagents.
- Utilizing a mouse model of Angelman syndrome with a deletion within the maternal allele of the *Ube3a* gene, neurons were dissociated and cultured *in vitro*.
- In separate wells, dissociated neurons were treated with ZFA AAV at DIV 7, with ASO at DIV12 or with a combination of AAV at DIV 7 and ASO at DIV12.
- Neurons were harvested for RT-qPCR analysis at DIV14.
- Results show comparability between the effects of ZFAs and ASOs with both increasing Ube3a expression in primary dissociated neurons from Angelman syndrome mice.



****P<0.0001, ***P<0.001
compared with negative control

Conclusions and next steps

- We have identified ZFAs capable of increasing both human and mouse UBE3A expression *in vitro*.
- Using Angelman syndrome disease neurons we show that the increase in UBE3A expression is being mediated by activation of the silenced paternal allele.
- For next steps, we plan to quantify any changes in Ube3a protein levels in mouse primary neurons following treatment with select ZFAs.
- In addition, we plan to perform an *in vivo* proof of concept study using translationally-relevant, phenotypic endpoints with lead surrogate ZFAs in a mouse model of Angelman syndrome.

References

1. Angela Mabb, Angelman syndrome: insights into genomic imprinting and neurodevelopmental phenotypes, *Trends Neurosci.* 2011

Disclosures

All authors are current or former employees of Sangamo Therapeutics

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