Investigating *SMARCE1* alternative 3'UTR mRNA isoform function in human ES cells

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Background

RNA Processing:

- RNA processing involves the maturation of newly transcribed mRNA to prepare it to exit the nucleus for translation
- Both alternative splicing (AS) and alternative polyadenylation (APA) are types of mRNA processing events that are regulated during cell development and differentiation
- microRNAs (miRNA) alter expression of genes through the binding mRNA at specific miRNA sites usually in the 3'UTR.

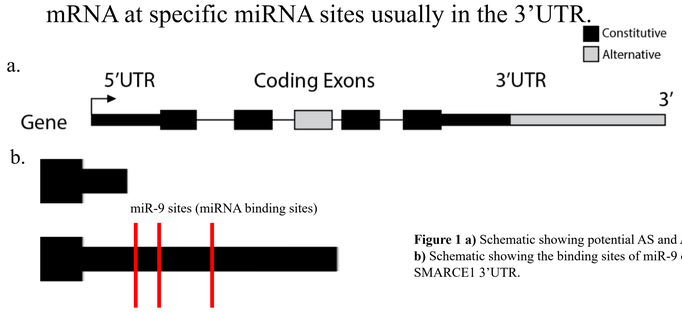

Figure 1 a) Schematic showing potential AS and APA events. **b)** Schematic showing the binding sites of miR-9 on SMARCE1 3'UTR.

Gene of Interest:

- *SMARCE1* is a key component of the BAF complex. The BAF complex functions as a chromatin remodeler. The *S*marce1/BAF57 protein is found in all canonical BAF (cBAF) complexes
- Smarce1 functions as a transcriptional cofactor with the role of guiding the complex to specific regions of the genome

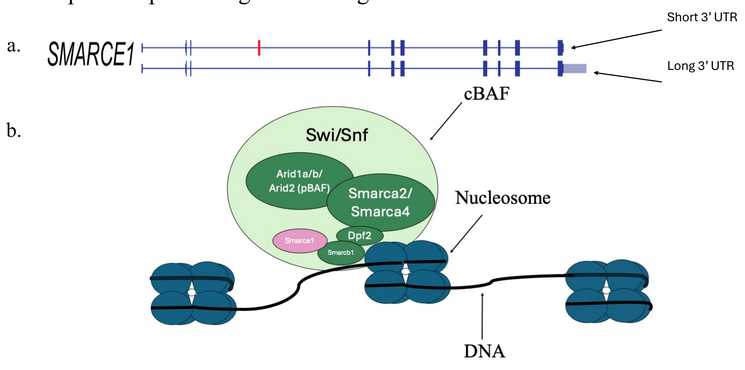

Figure 2 a) *SMARCE1* mRNA isoforms. **b)** Schematic of cBAF complex and function[2].

- Genetic mutations to *SMARCE1* and other BAF proteins can cause Coffin-Siris syndrome and implicated in meningioma

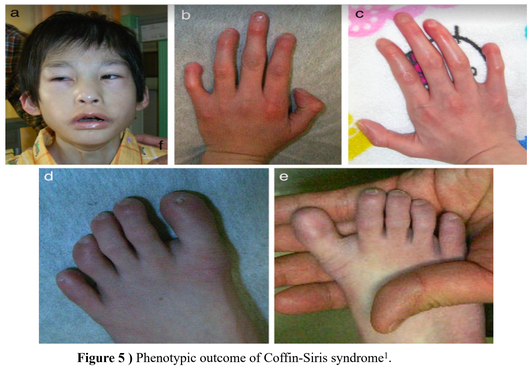

Figure 5) Phenotypic outcome of Coffin-Siris syndrome[1].

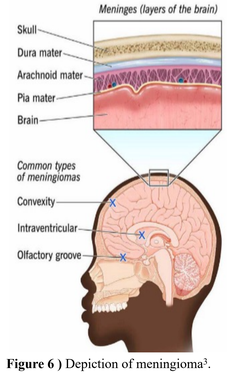

Figure 6) Depiction of meningioma[3].

Data

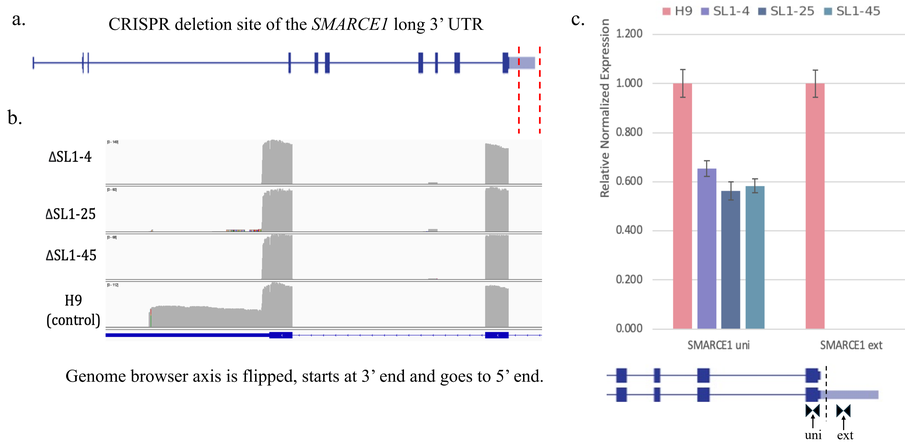

Figure 7 a) CRISPR deletion site on the long 3'UTR isoform of *SMARCE1*. **b)** Long-read RNA-sequencing data of the *SMARCE1* CRISPR knockdown, three separate deletion lines titled ΔSL-4, ΔSL-25, and ΔSL-45 as well as one control line H9. **c)** qPCR data using primers targeted to the uni and extended areas of the 3'UTR in *SMARCE1* CRISPR knockdown lines.

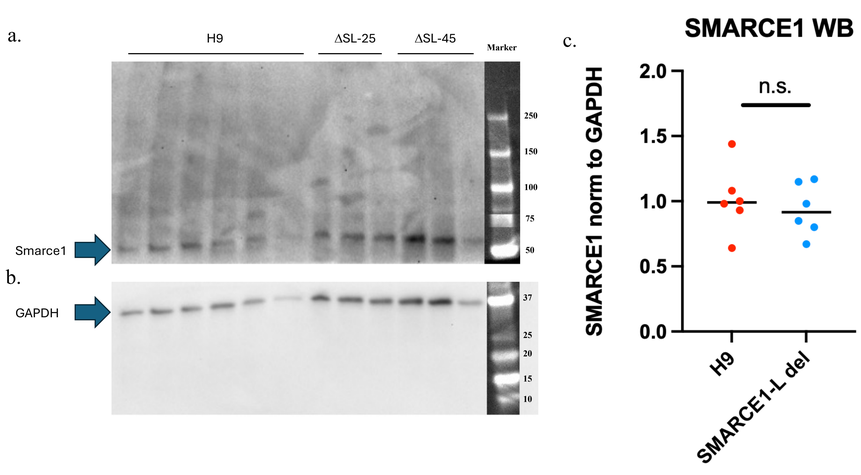

Figure 8 a) Western blot stained for Smarce1, *samples include* H9 1-6, ΔSL-25 1-3, and ΔSL-45 1-3. Smarce1 band appears at 55 kDa. **b)** Western blot stained for GAPDH, GAPDH appears at 36 kDa. **c)** Smarce1 band expression is normalized to their respective GAPDH band expression, deletion lines are then compared to the control lines.

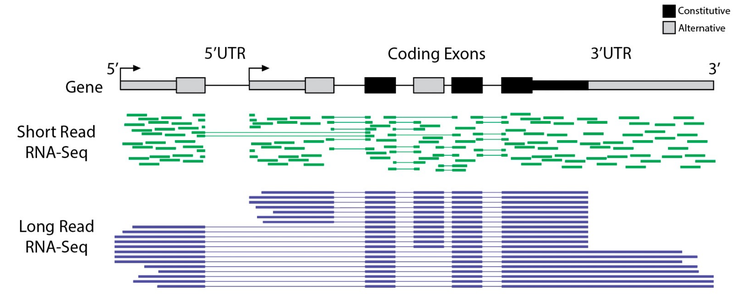

Figure 9: Schematic explaining the difference between short read and long read RNA-Sequencing[4].

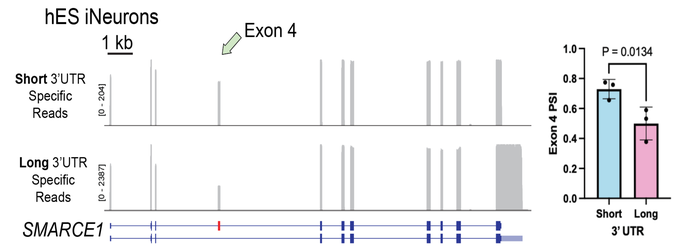

Figure 10 IGV coverage tracks showing the long and short 3' UTR isoforms of *SMARCE1,* showing a possible connection between exon 4 being expressed more in the short isoform when compared to the long isoform. Data generated by PL-Seq, a method for targeted long read sequencing on the Oxford Nanopore Technologies system.

Research Questions

- How does the deletion of the long 3'UTR in *SMARCE1* affect protein levels?
- How does *SMARCE1* long 3'UTR knockout (impact/affect) neuronal development and differentiation in human cells?
- Does AS and APA of *SMARCE1* showcase any "disease" phenotypes?

Future Directions

- Additional manipulation of the long and short 3'UTR in *SMARCE1* using CRISPR genome editing and shRNA knockdown
- Evaluate manipulated lines through ChIP-Seq
- Look at the effects on the phenotype of manipulated cells such as cell proliferation, morphology, and neural differentiation.

Conclusion

The western blot produces results that suggest that there are no significant differences in protein levels from the *SMARCE1* long isoform deletion line to the H9 control line in undifferentiated human embryonic stem cells.

Acknowledgements

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References

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4. Zhang, Z., Bae, B., Cuddleston, W. H. & Miura, P. Coordination of alternative splicing and alternative polyadenylation revealed by targeted long read sequencing. *Nat Commun* **14**, 5506 (2023).