CRISPR-Mediated Alteration of ARHGAP36 Expression in Human Medulloblastoma

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UROP
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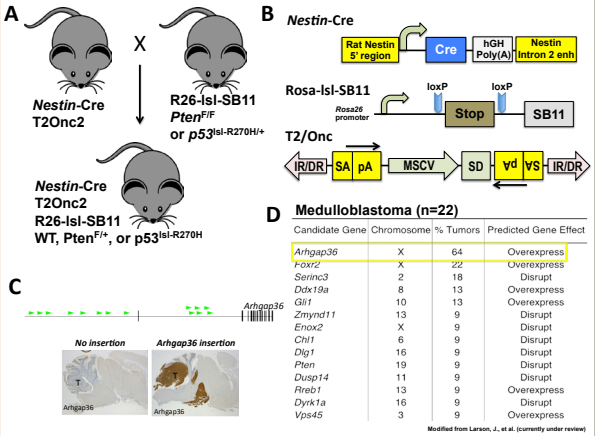
Medulloblastoma

- Most common pediatric CNS malignancy
- Targets the cerebellum during early development
- 400-450 new cases per year
- 4 Subgroups: WNT, SHH, Group 3, Group 4

Treatment	Side Effects
• Craniospinal irradiation	• Neurocognitive difficulties
• Surgical resection	• Blindness
• Aggressive chemotherapy	• Impaired spinal growth
	• Epilepsy

Arhgap36 and *Sleeping Beauty* Screen



Candidate Gene	Chromosome	% Tumors	Predicted Gene Effect
Arhgap36	X	64	Overexpress
Foxr2	X	22	Overexpress
Serinc3	2	18	Disrupt
Ddx19a	8	13	Overexpress
Gli1	10	13	Overexpress
Zmynd11	13	9	Disrupt
Enox2	X	9	Disrupt
Chl1	6	9	Disrupt
Dlg1	16	9	Disrupt
Pten	19	9	Disrupt
Dusp14	11	9	Disrupt
Rreb1	13	9	Overexpress
Dyrk1a	16	9	Disrupt
Vps45	3	9	Overexpress

Modified from Larson, J., et al. (currently under review)

Figure 1. ***Sleeping Beauty* insertional mutagenesis screen for MB**. A) Mouse model development driven by *Nestin*-cre recombinase generating mice with *Pten* or *p53* conditional deficiencies. B) Schematics of *Nestin*-Cre, SB11 transposon, and T2/Onc constructs. C) *Arhgap36* common insertion sites and MB stained cerebellum. D) *Arhgap36* emerges as top candidate driver gene for MB.

CRISPR/Cas Targeting *ARHGAP36*

Hypothesis: *ARHGAP36* deletion will reduce tumorigenic properties

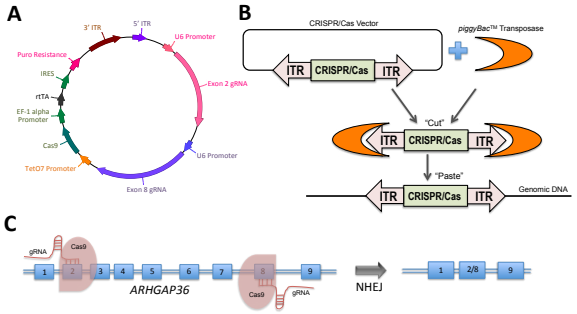


Figure 2. A) Map of CRISPR/Cas9 vector with dual gRNAs specific for *ARHGAP36* exon 2 and exon 8. B) Schematic of *piggyBac*™ transposition of CRISPR/Cas vector. C) Mechanism of gRNA directed Cas9 making double stranded breaks in *ARHGAP36*. Non-homologous end joining results in gene deletion or base pair mutations.

Generating Mutations/Deletions *In Vitro*

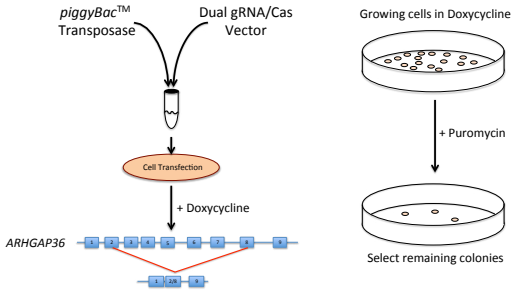


Figure 3. Schematic of transfection and selection techniques. *piggyBac*™ transposase and dual gRNA/Cas vector were lipofected into Ons76 and 293T cell lines. Doxycycline was added to transcribe the Cas9 enzyme and induce cutting. Puromycin was added to select for transfected clones. Clones were picked and analyzed for deletions and mutations

Results

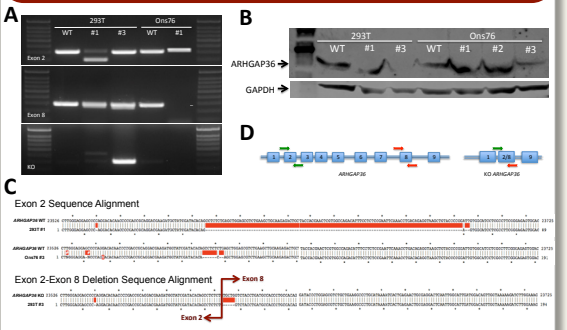


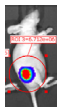
Figure 4. A) Agarose gel images of PCR for exon 2, exon 8 and KO flanking primers in 293T and Ons76 clones. B) Western blot showing reduced ARHGAP36 expression in 293T #3 and Ons76 #3 clones. C) Sequence alignment of 293T and Ons76 clones with WT or KO *ARHGAP36*. D) Schematic of primer binding pairs for PCR.

Discussion and Future Directions

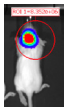
- We observed *ARHGAP36* mutation and deletions of various sizes in addition to reduced expression with this system.

Currently:

Future Directions



- *In vivo* flank injections in immunocompromised mice
- *In vivo* neonatal intracranial injections



Acknowledgments

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