A novel model of pediatric glioma of H3F3A mutant and TP53 mutant cerebral organoids

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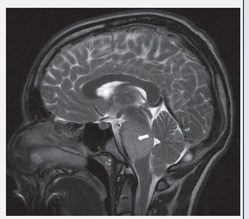
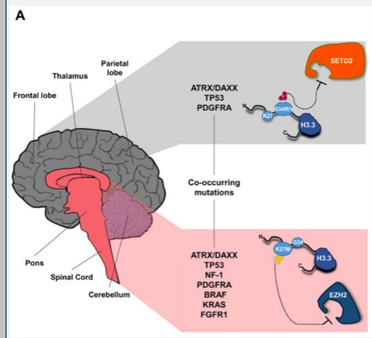
Diffuse Intrinsic Pontine Glioma (DIPG) Background

Diffuse Intrinsic Pontine Glioma (DIPG) is an **incurable childhood brainstem tumor,** affecting 200-400 children in the United States per year. Once diagnosed, the **only known treatment is radiation**, with death occurring in <12 months of initial diagnosis.

Resection is impossible due to its location and infiltrative growth. **Chemotherapeutic agents are ineffective** due to poor understanding of underlying molecular and cellular biology, and lack of *in-vitro* and *in-vivo* models for testing.



Mathew, Rutka, 2018



In patients with DIPG, genetic mutations of gain-of-function **K27M of Histone H3.3 gene (H3F3A)** and loss-of-function **P53 gene (TP53)** frequently co-occur.

Figure 1. Distribution and characteristics of H3.3-mutated gliomas model. Yeun, Kneopfler, 2013

Hypothesis

Combined TP53 and H3F3A mutations in human induced pluripotent stem cells (hiPSCs) will be a good model for DIPG development.

Experimental Design

(1) Use CRISPR/Cas9 to introduce **co-mutations of TP53 and H3F3A** in hiPSCs.

CRISPR/Cas9: Cells are transfected with a plasmid that codes for the **guide RNA**, **Cas9 protein**, and a selection marker for **puromycin resistance and/or hygromycin resistance.**

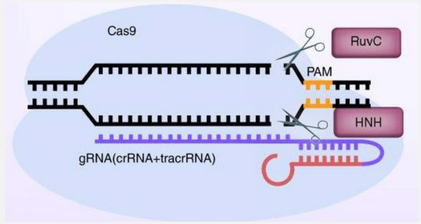


Figure 2. CRISPR/Cas9 Schematic. Chen and Knoepfler, 2016.

(2) Compare growth of these mutated hiPSCs in the form of **cerebral organoids**

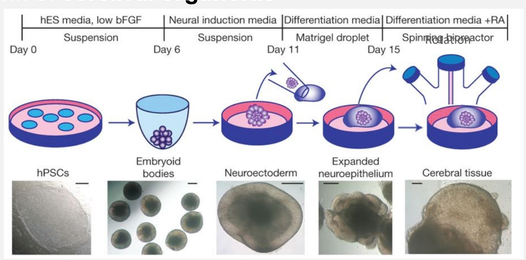


Figure 3. Brain organoid development timeline, Lancaster 2013.

Proliferation Analysis

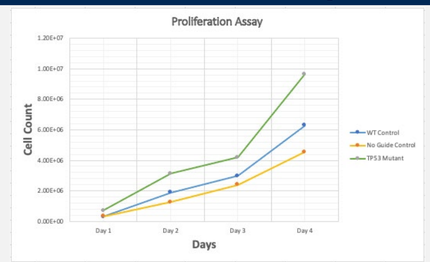


Figure 4. Proliferation Assay.

Analysis of TP53 Mutant Growth in Cerebral Organoid

TP53 mutant hiPSCs exhibit greater growth during cerebral organoid differentiation phase compared to control cell lines.

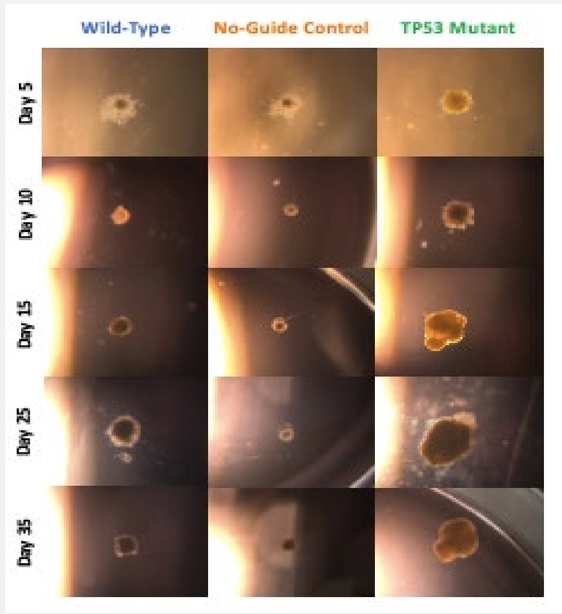
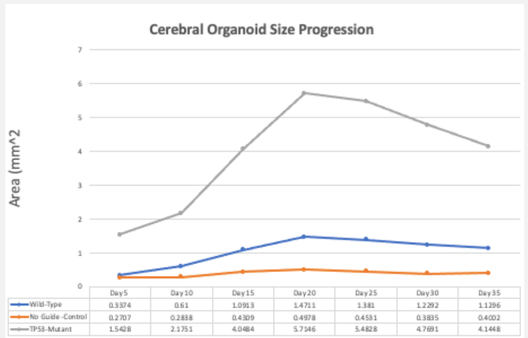


Figure 5. All images taken at 4x magnification



	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 35
Wild-Type	0.3374	0.61	1.0913	1.4711	1.381	1.2292	1.1296
No Guide -Control	0.2707	0.2838	0.4309	0.4978	0.4581	0.3835	0.4002
TP53-Mutant	1.5428	2.1751	4.0484	5.7146	5.4828	4.7691	4.1448

Figure 6. Size progression of cerebral organoid growth.

Conclusions

Larger sizes of TP53 mutant hiPSC of cerebral organoids and different cell cycle analysis compared to its non-mutated counterparts may show early signs of our intended model formation – we plan to **develop an *in-vitro* 3D model for DIPG** which can serve as a research tool.

Ongoing Directions

- **H3F3A mutation** on hiPSC.
- Analyze the molecular biology of TP53 mutant hiPSC for **RNA-seq** for IPSCs and organoid, **cell cycle, proliferation, apoptosis, differentiation, drug sensitivity,** and expression of **cancer markers**

References

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