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Targeting Cilia Initiation & Maintenance in SHH-Medulloblastoma

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Introduction

- Medulloblastoma (MB) is the most common malignant pediatric brain tumor and has four distinct molecular subtypes[1]
- Sonic Hedgehog (SHH) MB molecular subtype involves aberrant growth signaling between SHH mitogen and its receptors on primary cilium in cerebellar granule cells[2]
- We hypothesized that in mouse models genetically engineered to induce SHH-MB, additional genetic modifications dysregulating primary cilium would inhibit the cancer phenotype, either decreasing severity or preventing it altogether.

Methods

1. Establish breeding cages with genotypic combinations of:
   1. *Gpr161* conditional KO or CKO (Repressor of SHH-MB[3]).
   2. *Ptch1* ko (SHH receptor whose KO → SHH-MB).
   3. *Pcm1* ko (centriolar satellite protein critical for primary cilia).
2. PCR and gel electrophoresis to assess genotype.
3. Cerebellar dissection, cryo-slicing, antibody staining.
4. Immunofluorescence imaging using confocal microscopy.
5. Quantification using Fiji software.

Results

- Mice with *Gpr161* or *Ptch1* deletion develop SHH-MB and "persistent" external granular layers with increased cilia density.
- There is a large presence of BrdU (-) and CyclinD1 (-) cells with cilia.
- Heterozygous deletion *of Pcm1* +/ko does not diminish cancer phenotype, as a *Ptch1* +/ko; Pcm1 +/ko mouse demonstrated SHH-MB and increased cilia density.
- A *Gpr161* f/f; *Pcm1* ko/ko; *NestinCre* (DKO)mouse appears to rescue the cancer phenotype, as it did not develop tumor and had few cilia.
- We are currently crossing *Ptch1* (+/ko) with *Pcm1* +/ko mouse to generate *Ptch1* (+/ko) *Pcm1* ko/ko doubles to check for suppression of tumorigenesis.

Discussion, Limitations, & Future Directions

- In mice genetically predisposed to develop SHH-MB, destabilization of primary cilia may prevent cancer phenotype.
- If further validated, this could open an avenue for investigating therapeutic inhibition or downregulation of cerebellar primary cilia in human patients with genetic predisposition for SHH-MB.
- Limitations: low number of mice per category and human bias or error in quantification and measurement.
- Next steps: harvest more mice with *Ptch1* mutations and *Pcm1* DKO as *Ptch1* het produces more aggressive tumors, automate quantification process using Imaris software to limit human error.

Hypothesis

Genetically predispose mice to SHH-MB

↓

Destabilize primary cilia

↓

SHH-MB does not develop

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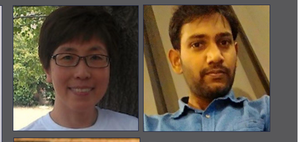
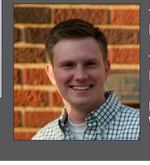
Thank you to all lab members:

Saikat Mukhopadhyay, M.D., Ph.D.





Top Left: **Sun-Hee Hwang, Ph.D.**

Top Right: **Vivek Palicharla, Ph.D.**

Bottom Left: **Kevin White, B.S.**

Images, Figures, and Table

Image 1 : SHH-MB GCs are ciliated

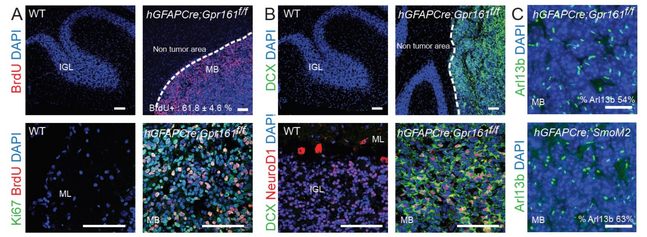


Image 2: Cilia shorten after losing centriolar satellites

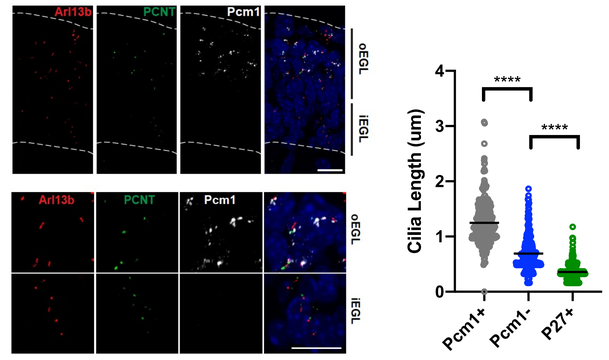


Table 1: PCM1 loss might prevent SHH-MB

Table 1: Effects of *Pcm1* Mutations in *Gpr161* CKO

	Pcm1 WT	*Pcm1* Het	*Pcm1* DKO
Tumor lesions Present?	Yes (n=4)	Yes (n=3)	No (n=1)
Average % Ciliated	53.67	50.86	5.93
Average Cilia Length	1.20 µm	1.05 µm	1.16 µm

References

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