**BACKGROUND**

- Pediatric high-grade glioma (pHGG) is the most common cause of pediatric cancer death.
- During normal development, the arginine methyltransferase PRMT5 serves a dual role of maintaining self-renewal in stem and progenitor cells and inducing their differentiation to a post-mitotic state.
- PRMT5 expression correlates with stemness and progenitor gene expression in pHGG cells. An shRNA screen of epigenetic regulators conducted in pediatric diffuse midline glioma (DMG) cell lines showed PRMT5 is essential for tumor cell growth.
- We hypothesized that PRMT5 epigenetically regulates self-renewal in pHGG.

**RESULTS**

**METHODS**

- We used lentiviral delivery of shRNA to knock down (KD) PRMT5 expression in five pediatric high-grade glioma (pHGG) cell lines – four diffuse midline glioma and one cortical diffuse glioma. We used a non-targeting shRNA as the control construct.
- **In vitro** studies included cell growth, bulk RNA-Seq and extreme limiting dilution (ELDA) assays.
- We orthotopically engrafted mice with patient-derived pHGG cells (PDX) with PRMT5 KD and tracked survival versus control tumors. We stained the PDX tumor samples with hematoxylin and eosin (H&E) and also for the proliferation marker Ki-67.

**CONCLUSIONS**

- **In vitro**, PRMT5 KD decreased the frequency of cells with self-renewal capacity and slowed the growth of pHGG cell lines. Transcritpicomic analysis showed that PRMT5 KD decreased expression of key self-renewal genes (PRMT5 KD/Ctrl). B. Geneset enrichment analysis of PRMT5 KD vs. ctrl shows decrease in self-renewal and increase in differentiation gene expression.
- **In vivo**, PRMT5 KD increased survival. Imaging studies and sample analysis showed that tumors forming from cells with PRMT5 KD were less aggressive and grew more slowly than control tumors.
- Decreased tumor growth associated with PRMT5 depletion demonstrates the importance of self-renewal maintenance to pHGG.
- PRMT5 appears to play an important role in self-renewal maintenance in both H3K27M mutant and H3-wt pHGG. Further exploration of PRMT5’s mechanism of self-renewal maintenance will enhance understanding of pHGG oncogenesis and growth, potentially leading to the development of better therapies.

**IMPLICATIONS**

- We are completing ChiP-Seq experiments to determine whether and how PRMT5 epigenetically controls self-renewal.
- The self-renewal phenotype may present an opportunity for new therapeutic interventions in pHGG, focused on eliminating conditions that enable self-renewing populations to grow.
- PRMT5 is a potential therapeutic target in pHGG. We are working with clinically approved PRMT5 inhibitors as well as novel inhibitory strategies, such as substrate depletion, to determine whether PRMT5 inhibition produces similar results to those seen with PRMT5 KD.
- We plan to also study combination therapies combining PRMT5 inhibition with radiotherapy, self-renewal depletion, and inhibition of other targets.

**DISCLOSURES**

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