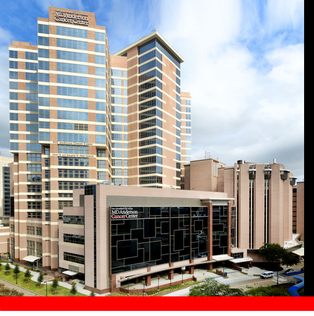

Study of protein arginine methyltransferase 6 in medulloblastoma

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Purpose

Protein arginine methyltransferase 6 (PRMT6) was identified in a gain of function screen in which it enhanced proliferation of high REST expressing medulloblastoma cells. To better understand this interaction and its role in tumorigenesis, we studied the expression of PRMT6 in medulloblastoma and how knocking down this protein *in vitro* affects cancer cell viability. Potential therapeutic inhibitors of PRMT6 were tested for cytotoxicity in medulloblastoma cell lines.

Background

Medulloblastoma is the most common malignant brain tumor in children and a major cause of cancer related childhood mortality. Current treatments have drastic side effects that can last throughout a patient's lifetime. The search for new treatments requires a better understanding of medulloblastoma biology. The sonic hedgehog (SHH) subgroup of medulloblastomas overexpress RE1-silencing transcription factor (REST), which represses neuronal differentiation genes and drives tumorigenesis. REST elevation in cerebellar granule progenitor cells (CGNPs) increased proliferation and decreased telomere length (1). The latter is a trigger for senescence and apoptosis; however, these cells escape both processes. To better understand these mechanisms, we performed a gain of function screen with a library of epigenes. Protein arginine methyltransferase 6 (PRMT6) was identified as a high-priority REST-interacting protein. PRMT6 is known to regulate the expression of genes involved in senescence (2). The availability of PRMT6 specific inhibitors for evaluation in pre-clinical studies was an added criterion for our focus on PRMT6. Our hypothesis is that PRMT6 prevents senescence in REST-driven SHH medulloblastomas.

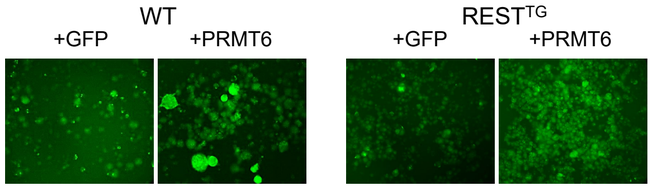

Fig. 1 After 18 days, REST^{TG} progenitor cells overexpressing PRMT6 have enhanced proliferation compared to WT.

Methods

Published transcriptomic databases were mined for PRMT6 and REST expression and survival curves were generated. The brain sections of mice harboring patient derived xenografts (PDX) were stained with hematoxylin and eosin (H&E) to ensure tumors were present. Immunohistochemistry was performed to study the expression of PRMT6 in DAOY and DAOY high REST tumors. PRMT6 was knocked down in medulloblastoma cell lines using siRNA and cell viability was measured using MTT assays. qPCR will be done to confirm reduction of PRMT6 expression. In pre-clinical studies, MTT assays were performed to determine the IC50s of PRMT6 inhibitors (Licochalcone A, EPZ020411, and MS117) against a panel of medulloblastoma cell lines.

Results

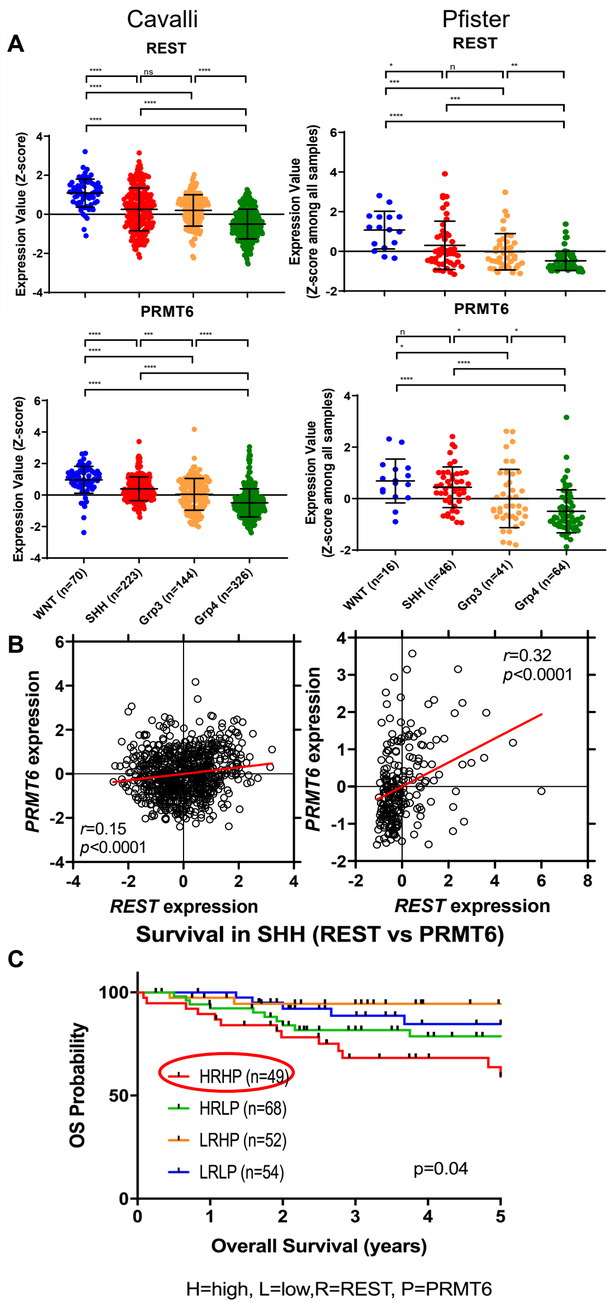

Fig. 2 A. REST and PRMT6 expression were evaluated in the four molecular subgroups of medulloblastoma (WNT, SHH, Grp 3, and Grp 4) based on data collected from Cavalli and Pfister public databases. **B.** REST and PRMT6 expression Z-scores were compared to see if there was a correlation. **C.** The survival curve shows the probability of survival of patients with different levels of REST and PRMT6 expression in tumors. (3,4)

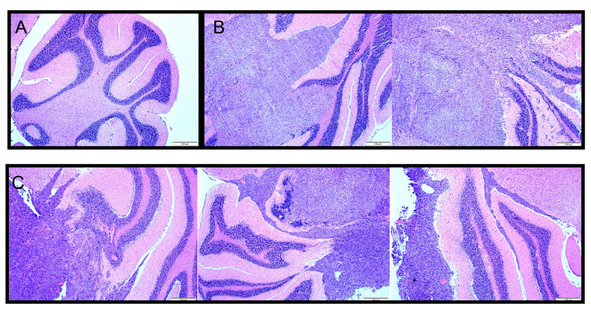

Fig. 3 H&E staining of **A.** wild type mouse cerebellum, **B.** DAOY xenograft tumors, and **C.** DAOY high REST xenograft tumors.

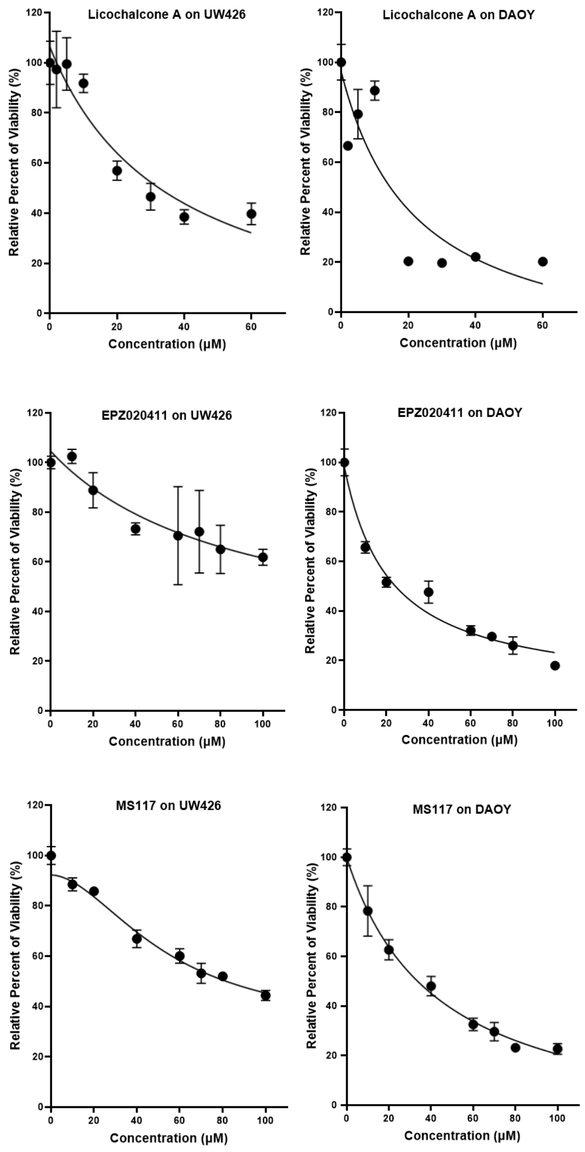

Fig. 4 MTT assay data for SHH cell lines (UW426 and DAOY) treated with three PRMT6 inhibitors.

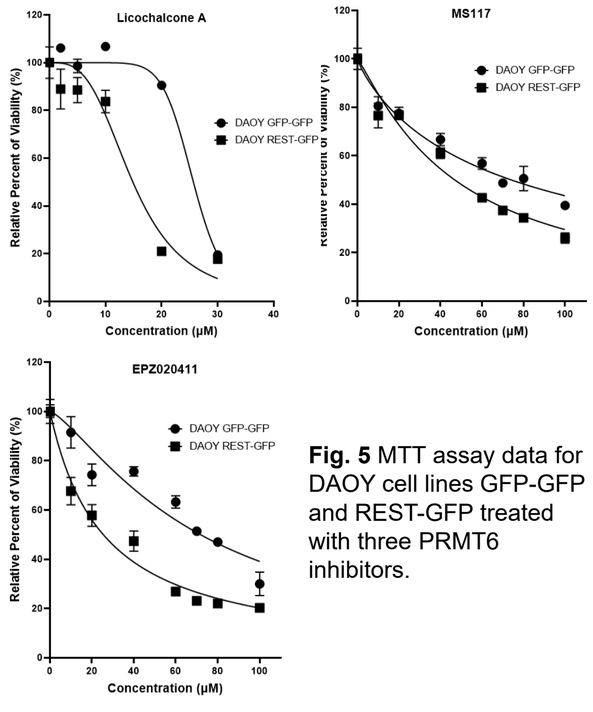

Fig. 5 MTT assay data for DAOY cell lines GFP-GFP and REST-GFP treated with three PRMT6 inhibitors.

Table 1 Half maximum inhibitory concentrations of PRMT6 inhibitors tested in SHH cell lines where GG and RG represent DAOY GFP-GFP and REST-GFP respectively.

IC50 Values (μM)	UW426	DAOY	GG	RG
Licochalcone A	36.15	21.98	25.66	14.88
EPZ020411	87.68	21.55	71.83	25.47
MS117	54.01	42.65	74.32	47.00

Discussion

PRMT6 overexpression in cells with elevated REST expression enhanced proliferation and may contribute to tumorigenesis. To better understand the relationship between REST and PRMT6 in medulloblastoma, expression data was collected from two public patient databases and analyzed. REST and PRMT6 expression were elevated in both WNT and SHH driven medulloblastoma tumors. There was also a weak, positive correlation between REST and PRMT6 expression values. Co-elevation of REST and PRMT6 was associated with poor patient prognosis. PRMT6 expression will also be evaluated in a PDX mouse model of SHH medulloblastoma. H&E staining located tumors in the brains of mice xenografted with human medulloblastoma cell lines (DAOY and DAOY high REST). Experiments are ongoing to evaluate the expression of PRMT6 in tumors and the effect of PRMT6 knockdown on viability of SHH cell lines. MTT assays revealed the cytotoxicity of PRMT6 inhibitors in SHH medulloblastoma cells in vitro. The UW426 cell line was more resistant to each inhibitor than DAOY, indicated by the greater IC50 values. DAOY REST-GFP was more sensitive to each inhibitor compared to DAOY GFP-GFP. Research is ongoing to understand whether PRMT6 is a driver of medulloblastoma and could be targeted for future therapies.

Conclusions

We have identified a novel genetic interaction between REST and PRMT6 in medulloblastoma. If PRMT6 knockdown significantly decreases cell viability and parallels our findings from pharmacological studies with PRMT6 inhibitors, we will have provided the foundation for further mechanistic and pre-clinical studies focused on investigating the role of PRMT6 in REST-driven SHH medulloblastomas and the feasibility of targeting it for treatment.

Acknowledgements

Partnership for Careers in Cancer Science and Medicine Program

References

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