**Introduction**

- Medulloblastoma (MB) is the most common tumor of the central nervous system in children with high metastasis rate and ineffective therapies.
- We previously found the upregulated expression of the RE1-Silencing Transcription Factor (REST), a repressor of neurogenesis, to be associated with poor survival in patients with Sonic Hedgehog (SHH) subgroup of MBs.
- Through a loss of function screen, we identified DNA methyltransferase 1 (DNMT1) as a high-priority candidate that is critical for the viability of tumor cells in the context of REST elevation in SHH-MBs.

**Hypothesis:** We assume that REST is altering DNA methylation and gene expression in SHH-MB.

![DNMT1](image)

**Figure 1:** A loss of function screen identifies DNMT1 as a high-priority candidate that is critical for the viability of tumor cells in the context of REST elevation in SHH-MBs. The knockdown of DNMT1 blocks cell growth of high-REST cells.

**Methods**

To detect the effects of REST upregulation on DNA methylation status and the overall survival, we analyzed a publicly available database and divided the SHH-MBs into two groups based on REST levels expression (high-REST and low-REST). Overall survival graphs are generated for both groups as well as volcano plots showing the DNA methylation differences and REST levels at different genomic locations. We also detected the correlation between gene expression and DNA methylation changes. Genes with significant DNA methylation changes were chosen for pathway analysis. Chosen genes are validated in human MB cell lines as well as in mouse model.

**Results**

**Overall Survival in SHH-MB patients**

![Overall Survival in SHH-MB patients](image)

**Figure 3:** Overall survival of Sonic Hedgehog Medulloblastoma patients with High- and Low-REST levels over 20 years. High-REST patients (n=22) have worse overall survival probability compared to that of low-REST patients (n=40) (p=0.05).

**DNA methylation differences in context of REST levels**

![DNA methylation differences in context of REST levels](image)

**Figure 4:** Volcano plots showing the DNA methylation differences based on REST levels in SHH-Medulloblastoma at all sites in three different genomic locations. Each red dot on the positive scale represents a hypomethylated CpG site in high-REST SHH-MBs. Blue dots on the negative scale represents a hypermethylated CpG site in high-REST SHH-MBs. Blue dot on the positive scale represents a hypermethylated CpG site in high-REST SHH-MBs. The knockdown of DNMT1 blocks cell growth of high-REST cells.

**Gene expression differences in context of REST levels**

![Gene expression differences in context of REST levels](image)

**Figure 5:** Volcano plots showing the DNA methylation differences between high-REST SHH-MBs and low-REST SHH-MBs at the promoter region in three different genomic locations. CpG sites at the promoter in CpG island (CGI) showed a lower hypermethylation percentage than that inshore, and non-CGI (0.8% vs. 0.1% and 1.8%) respectively; p<0.05; DNA methylation difference >10%. A. DNA methylation differences and REST levels at the promoter in CGIs. B. Promoter at shore and shelf. C. Promoter at non-CGIs.

**Correlation between gene expression and DNA methylation**

![Correlation between gene expression and DNA methylation](image)

**Figure 6:** Volcano plot showing the gene expression changes and REST levels in 2026 genes. Red dots on the positive scale represent genes with high REST and gene expression upregulation. Blue dots on the negative scale represent genes with Low REST and gene expression downregulation.

![Correlation between gene expression and DNA methylation](image)

**Figure 7:** Correlation graph representing a non-significant correlation between gene expression differences and DNA methylation differences in high- and low-REST CpG sites at the promoter in 9827 genes. 41 genes in blue represent hypermethylation with gene expression upregulation; 58 genes in blue represent hypermethylation with gene expression downregulation.

**Conclusion**

- REST elevation in SHH-MBs is associated with poor survival. REST elevation promotes tumor cell proliferation and the knockdown of DNMT1 blocks REST-dependent cell growth.
- Compared to tumors with low REST expression, samples with higher REST expression exhibit hypermethylation at the promoter, gene body and intergenic locations.
- There is a negative correlation between gene expression and DNA methylation in high-REST genes at the transcription start site in the promoter region that is still ongoing.
- Hypermethylated genes play a role in development and differentiation processes of cancer.

**Future Work**

- RNA sequencing to identify the disrupted events of the initiation, splicing, and elongation of transcription in high-REST SHH-MB.
- Validation in human MB cell lines (DOAY, UW228, and UW426) through bisulfite pyrosequencing and quantitative RT-PCR.
- Validation in SHH-MB mice model (RESTTg and WT).

**References**

- Cavalli et al., Cancer Cell. 2017;31(6):737-754.

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