RE1- Silencing Transcription Factor (REST) and DNA Methyltransferase 1 (DNMT1) Interaction in Medulloblastoma

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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.

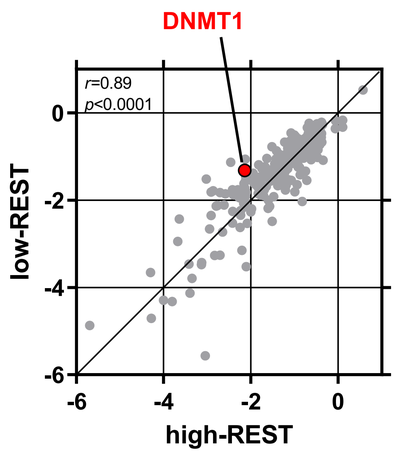
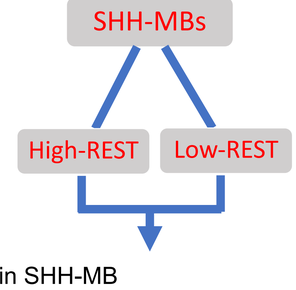


Figure 1: A loss of function screen identifies DNMT1 as a high priority candidate that is critical for the viability of tumor cells in the context of REST elevation in SHH-MBs. The knockdown of DNMT1 blocks cell growth of high-REST cells.

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 mice model.



1- Overall Survival in SHH-MB
2- DNA methylation differences in REST context
3- Gene expression differences in REST context
4- Validation in human MB cell lines (DAOY, UW228, and UW426)
5- Validation in overlapped genes in mice model (REST^{TG} and WT)

Figure 2: Flow chart showing the research strategy. Patient data was divided into two groups based on the average of REST mRNA levels.

Results

Overall Survival in SHH-MB patients

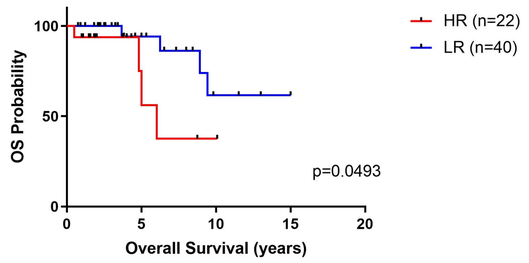


Figure 3: Overall survival of Sonic Hedgehog Medulloblastoma patients with High- and Low-REST levels over 20 years. High-REST patients (n=22) have worse overall survival probability compared to that of low-REST patients (n=40) (p<0.05).

DNA methylation differences in context of REST levels

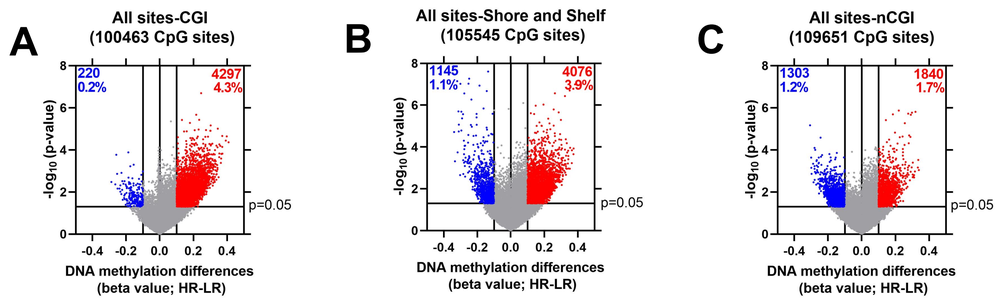


Figure 4: Volcano plots showing the DNA methylation differences based on REST levels in SHH-Medulloblastoma at all sites in three different genomic locations. Each red dot on the positive scale represents a hypermethylated CpG site in high-REST SHH-MBs. Blue dot on the negative scale represents a hypomethylated CpG site in high-REST SHH-MBs. CpG sites in CGIs (CpG islands) are more hypermethylated when REST levels are high than in shore, shelf and non-CGI (4.3% vs 3.9% and 1.7%) respectively; p<0.05; DNA methylation difference > |10%|. **A.** DNA methylation differences between high-REST SHH-MBs and low-REST SHH-MBs at CpG island in CGIs. **B.** CpG sites at shore and shelf. **C**. CpG sites at non-CGIs.

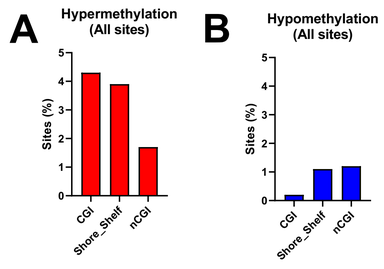


Figure 5: Bar graph of the percentage of DNA methylation of all sites at three genomic locations (CGI, shore and shelf, and non-CGI) of high-REST SHH-MB patients. **A.** CpG sites at CGIs showed higher ratio of hypermethylated sites than at shore and shelf and non-CGIs. **B.** CpG sites at non-CGIs are prone to hypomethylation compared to CGIs and shore and shelf.

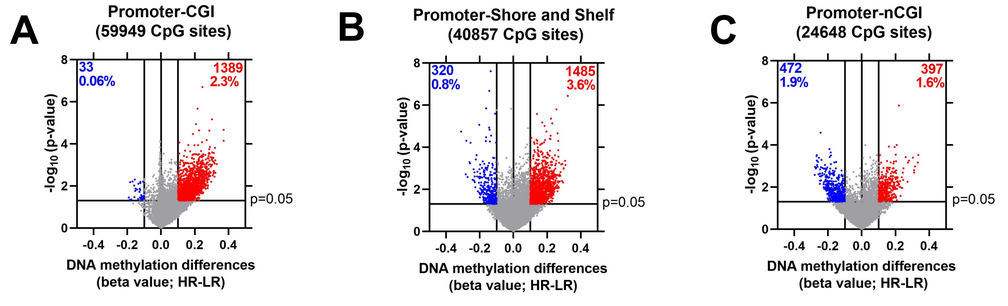


Figure 5: Volcano plots showing the DNA methylation differences between high-REST SHH-MBs and low-REST SHH-MBs at the promoter region in three different genomic locations. CpG sites at the promoter in CpG island (CGIs) showed a lower hypomethylation percentage than that in shore, shelf, and n-CGI (0.06% vs 0.8% and 1.9%) 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.

Gene expression differences in context of REST levels

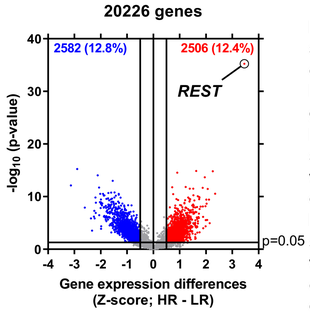


Figure 6: Volcano plot showing the gene expression changes and REST levels in 20226 genes.
Red dots on the positive scale represent genes with high REST and gene expression upregulation.
Blue dots on the negative scale represent genes with Low REST and gene expression downregulation.

Correlation between gene expression and DNA methylation

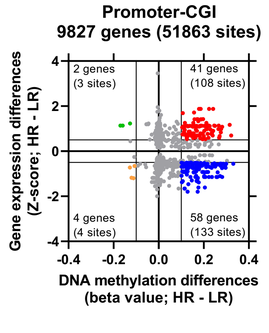


Figure 7: Correlation graph representing a non-significant correlation between gene expression differences and DNA methylation differences in high- and low- REST CpG sites at the promoter in 9827 genes. 41 genes in red represent hypermethylation with gene expression upregulation; 58 genes in blue represent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

Pathway Analysis

Diseases and disorders name	p-value range	# Molecules
Cancer	3.39E-02 - 1.25E-07	57
Organismal Injury and Abnormalities	3.39E-02 - 1.25E-07	58
Endocrine System Disorders	3.39E-02 - 1.91E-06	51
Cardiovascular Disease	2.71E-02 - 9.35E-06	13
Neurological Disease	3.39E-02 - 9.35E-06	43

Table 1: Pathway analysis showing the name of the diseases and disorders as well as the p-value range and number of molecules that the 58 chosen genes with downregulated expression and DNA hypermethylation are involved in.

Molecular and cellular functions	p-value range	# Molecules
Cell-To-Cell Signaling and Interaction	3.39E-02 - 5.90E-07	13
Molecular Transport	3.15E-02 - 5.90E-07	21
Small Molecule Biochemistry	3.15E-02 - 5.90E-07	16
Cell Morphology	2.91E-02 - 8.85E-05	8
Cellular Assembly and Organization	3.39E-02 - 8.85E-05	11

Table 2: Pathway analysis showing the molecular and cellular functions as well as the p-value range and number of molecules that the 58 chosen genes with downregulated expression and DNA hypermethylation are involved in.

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 (REST^{TG} and WT).

References

Dobson et al., *Science Signaling*. 2019;12(565):eaan8680.
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Acknowledgment

Texas Medical Center Summer Research and Internship Program.
MD Anderson Summer Experience - University Outreach Program.