Frequent MTAP Deletion shapes the DNA Methylome and Drug Sensitivity in Glioblastoma Cells

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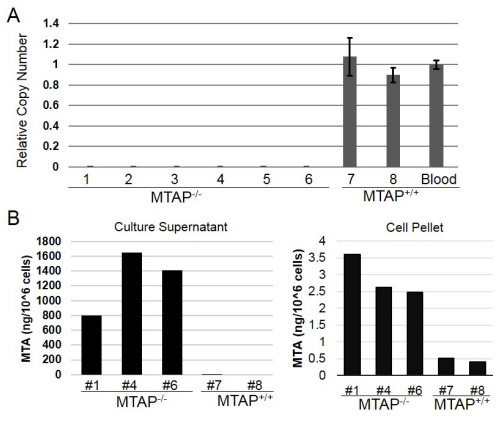
Introduction

Glioblastoma (GBM) is the most common and deadly primary malignant brain tumor, and homozygous deletion of the methylthioadenosine phosphorylase (*MTAP*) gene occurs in 50% of all GBM patients. The pathogenic consequence of *MTAP* deletion in GBM, however, remains unclear. MTAP is a metabolic enzyme involved in purine salvage, and MTAP loss results in the accumulation of its metabolite, methylthioadenosine (MTA), a known methyltransferase inhibitor. Through in vitro and in vivo studies, we found that MTAP loss profoundly alters tumor biology and is associated with epigenetic reprograming, altered cell identity, and differential sensitivity to therapeutic agents, suggesting this frequent genetic alteration could provide a valuable focus for targeted therapy.

MTAP expression and MTA accumulation in GBM cells

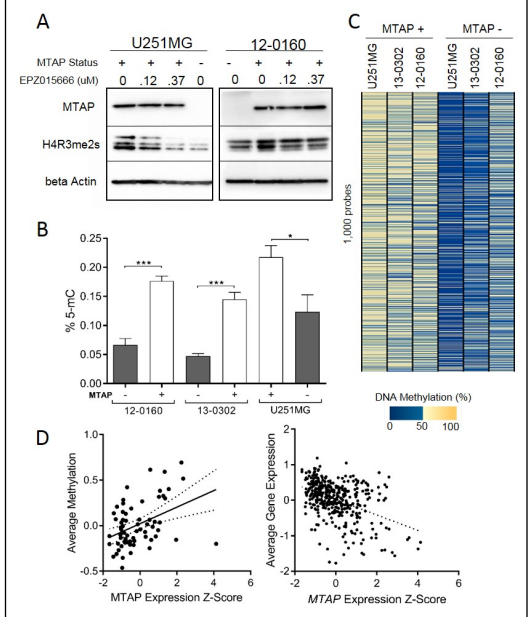


(A) Relative MTAP expression was quantified in patient-derived GBM cells by RT-PCR. (B) MTA was measured by LC-MS/MS in culture media (left) and cell pellets (right).

Methods

We measured the effects of MTAP loss on isogenic GBM cell lines using DNA methylation (illumina) and gene expression (Affymetrix) arrays as well as quantitative PCR and pyrosequencing. We generated orthotopic xenografts to evaluate tumorigenicity and tested the response of tumor cells to select compounds *in vitro* and *in vivo*.

Impact of MTAP status on epigenetics

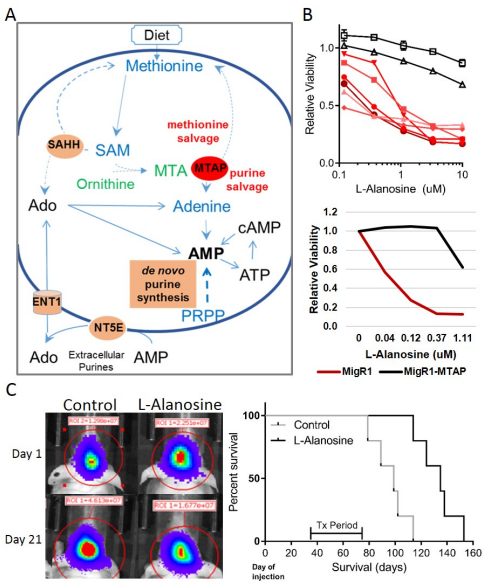


(A) MTAP status and PRMT5 inhibition alter histone methylation. (B) Global 5-mC ELISA shows DNA hypomethylation in MTAP-null cells (n 6). (C) Heatmap showing DNA hypomethylation at the most differentially methylated DNA loci between isogenic cell pairs (MethylationEPIC 850k array). (D) Patient data (TCGA) shows decreased methylation (left) and increased gene expression (right) with MTAP deficiency.

Results

MTAP loss resulted in epigenomic reprogramming with differential gene expression and enhanced tumorigenesis in GBM cells and patient samples. Furthermore, MTAP loss alters the cellular response to purine starvation, temozolomide, and to selected epigenome-modulating compounds.

MTAP null cells are sensitive to purine deprivation

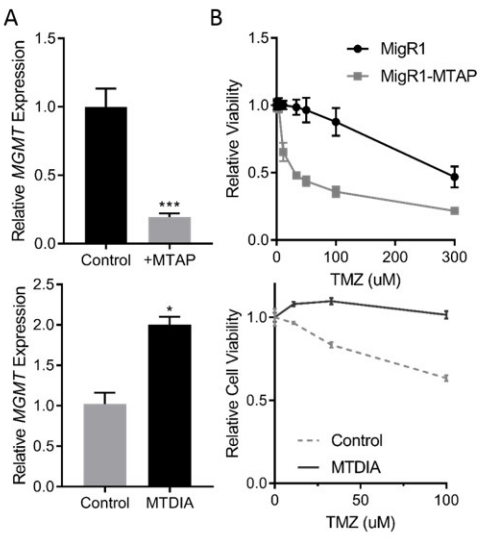


(A) MTAP pathway (B, C) MTAP null cells are sensitive to purine synthesis inhibition (B) in vitro (red=MTAP null, black=MTAP+)and (C) in orthotopic nude mouse xenografts in vivo.

Conclusions

These findings suggest a broad impact of *MTAP* deletion on the epigenetic landscape and implicate MTAP loss as a contributing factor in the pathogenesis of GBM and as a viable focus for designing targeted therapies.

MTAP deficient cells are TMZ resistant



(A) MGMT expression measured by RT-PCR (B) Cell viability 5 days after TMZ treatment measured by CCK8.

Learning Objectives

By the conclusion of this presentation, participants should be able to: 1) know the frequency and consequences of MTAP deletion in GBM, 2) recognize the clinical value of genomic tumor characterization, 3) Consider novel targeted therapy approaches that exploit tumor cell vulnerabilities.

References

Hansen LJ, et al., MTAP loss promotes stemness of glioblastoma cells and confers unique susceptibility to purine starvation. In Revision at Cancer Research.

Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462-77