Increased HEY1 Expression Contributes to Glioma Progression and Promotes Glioma Cell Growth

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Introduction

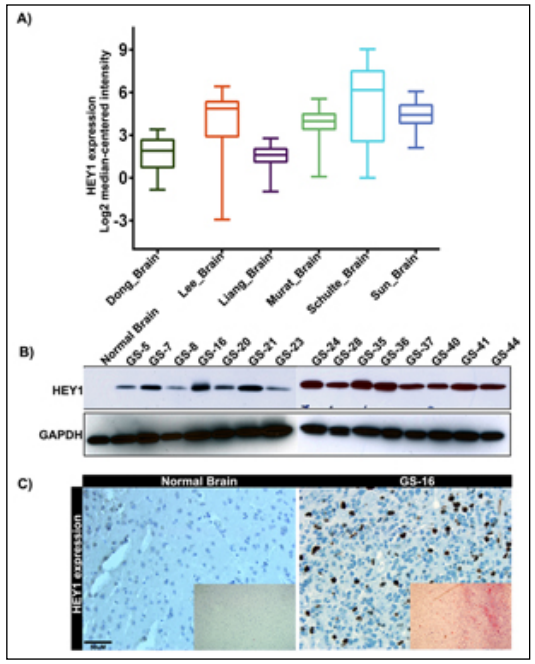
Glioblastoma multiforme (GBM) is a highly aggressive brain tumor characterized by increased necrosis and intense resistance to therapy. Although, little is known about the molecular mechanisms that underlie glioblastoma formation, a number of signal transduction routes, such as the Notch, EGFR and p53 pathways, seem to play an important role in the formation/maintenance of GBM.

Methods

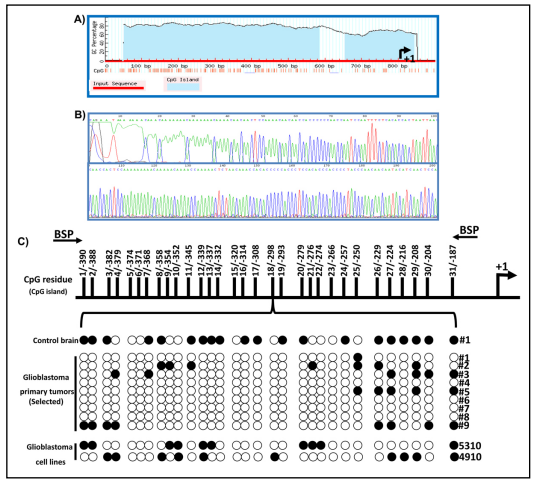
Notch signaling is aberrantly activated in GBM and orchestrates the malignant traits of GBM, but current knowledge regarding the downstream players of Notch in GBM remains limited. Recently we have identified that Notch effector HEY1 is highly upregulated in hGBM (human) surgical biopsies and its overexpression appears to be correlative with high-grade glioma. Our immunoblot experiments revealed that HEY1 expression significantly correlated with O6-alkylguanine DNA alkyltransferase (MGMT) levels in hGBM specimens.

Results

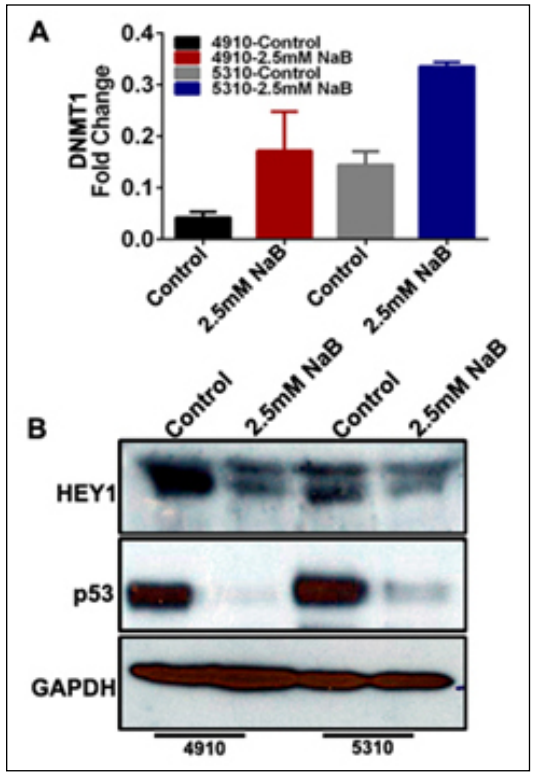
The inhibition of HEY1 reduced invasive, migratory and proliferative abilities of GBM cells, in vitro. Furthermore, our experiments with TF -TF; TF-DNA arrays conducted using recombinant HEY1 showed its interaction with multiple transcription factors emphasizing its importance in GBM, highlighting HEY1 as a potential therapeutic target that can be evaluated in GBM.



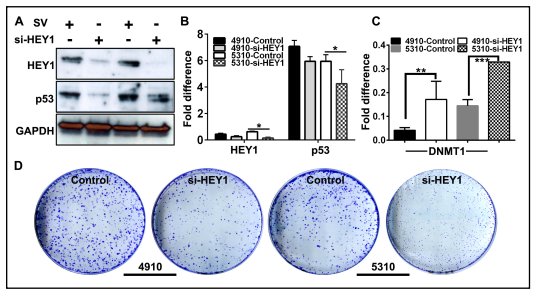
HEY1 is overexpressed in hGBM. (A) Whisker plots of HEY1 gene expression catalogued within the OncomineTM database(B) Immunoblotting analysis of normal brain and of GBM samples (GS), (n=15) for detecting HEY1 expression (C) Immunohistochemical analysis of normal brain and GS-16 to detect HEY1 using DAB staining (Bar = 50μM). Inset images represent Hematoxylin and Eosin staining of the respective specimens.



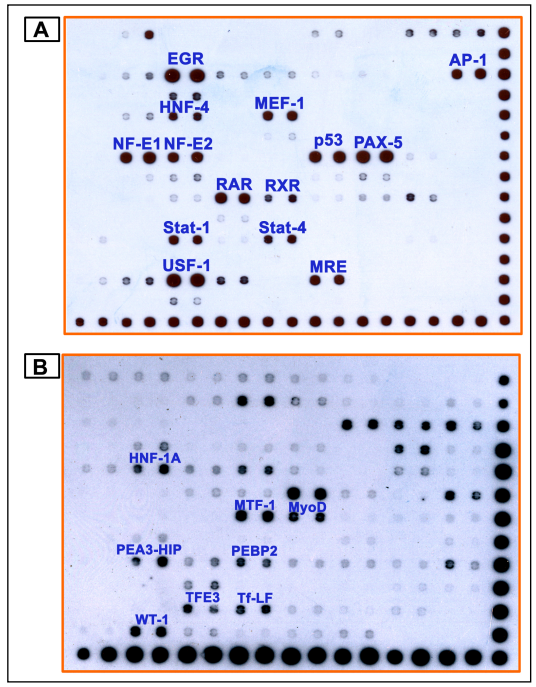
CpG islands of the HEY1 gene determine demethylation in GBM. (A) CpG islands on promoter region (left of arrow) of the HEY1 gene(B)sequencing analysis (C) Areas of methylation are denoted as as black circles.



Sodium Butyrate (NaB) inhibits expression of HEY1/p53 (A) qRT-PCR to check the expression levels of DNA methyltransferase 1 (DNMT1) on the mRNA of 4910, 5310 control and their treatment of cells with 2.5mM NaB (B) Immunoblot analysis of HEY1 and p53 in control and 2.5mM NaB treated cells



si-HEY1 reduces phenotypes associated with cancer in vitro. (A) Immunoblot analysis of HEY1 and p53 levels in SV and si-HEY1 treatments (SV = Scrambled vector; si-HEY1 = small interfering RNA for HEY1) (B & C) qRT-PCR analysis of si-HEY1 treatment to detected the levels of HEY1, p53 and DNMT1 (D) Clonogenic assay



Potential interaction of HEY1 with multiple transcription factors. (A) TF -TF array was performed using recombinant HEY1 and anti-HEY1 antibody as per the manufacturer's instructions (Affymetrix, CA; #MA1210) and potential HERP2-interacting proteins are highlighted.

Conclusions

- Within glioblastoma, HEY-1 is found to be overexpressed when compared to its normal brain counterparts.
- Demethylation of the CpG islands within the promoter region of HEY1 occurs in GBM, which is a possible mechanism of overexpression of the gene.
- Treatment with sodium butyrate (NaB) is shown to lower expression of HEY-1 and p53 while diminishing cancer phenotypes.
- si-HEY1 additionally reduces HEY1 and p53 expression in GBM, also leading to a reversion of proliferative abilities in vitro and resumed adherence to mitotic checkpoints.
- HEY1 interacts with a number of transcription factors and other proteins, suggesting the involvement of this molecule in a number of signaling cascades that contribute to the phenotype of GBM.