TGF-β1/Smads-induced TET3 demethylates Otx2 superenhancer to promote Group3 medulloblastoma tumor growth

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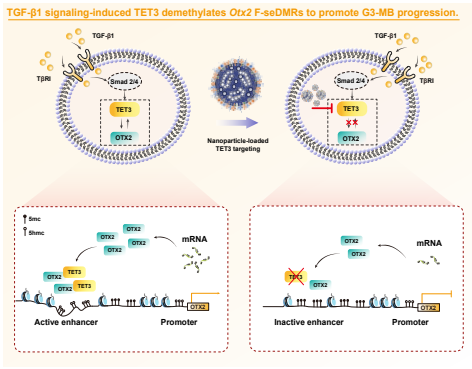
Study Overview

Background

While DNA methylation patterns have been widely employed for medulloblastoma (MB) classification, limited understanding of epigenetic regulatory bases of superenhancer (SE) hinders development of potential interventions for MB.
Distinct DNA methylation patterns were identified across four MB subgroups. Particularly, in G3-MB, three tumor cell populations, including progenitor-like, cycling, differentiated neuronal-like tumor cells, were captured. DNA hypomethylation of functional SE differentially methylated regions (hypo-F-seDMRs) exerted a negative correlation with CA thus affecting gene expression in the three cell populations, which was also associated with embryonic cerebellum development. Otx2 hypo-F-seDMRs served as an independent prognostic indicator in G3/4-MB patients. Important OTX2 self-binding sites were discovered within the Otx2 hypo-F-seDMRs region.

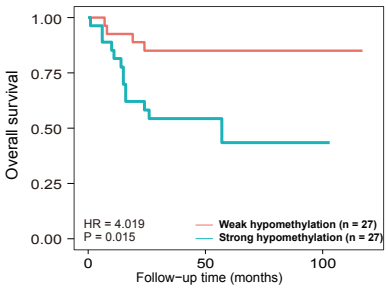
Results

Deletion of the binding motif at accessible sites of Otx2 SE reduced its own expression and abrogated tumor growth, indicative of the auto-regulation. Mechanistically, Ten-Eleven translocation 3 (TET3), specifically recruited by OTX2 and triggered by TGF-β1/Smad2 signaling, demethylated OTX2 binding sites and open chromatins, to sustain tumor proliferation and stemness. Finally, LNPs-coated siTET3 or Bobcat339 significantly inhibited Group3 MB tumor growth.



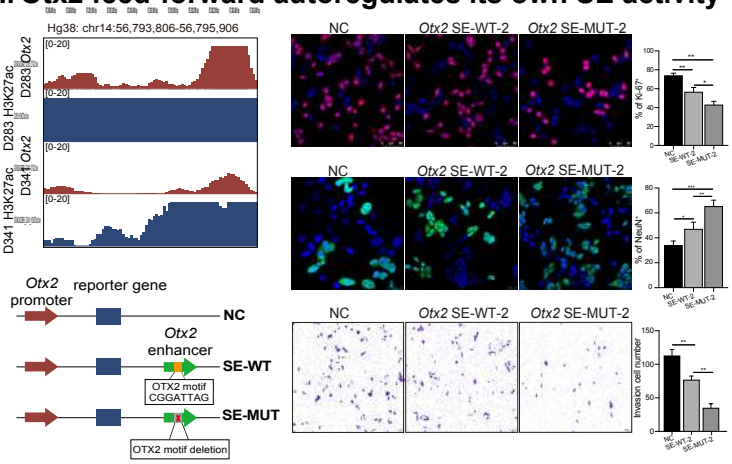
Results

Otx2 hypo-F-seDMRs served as an independent prognostic indicator in G3/4-MB patients.



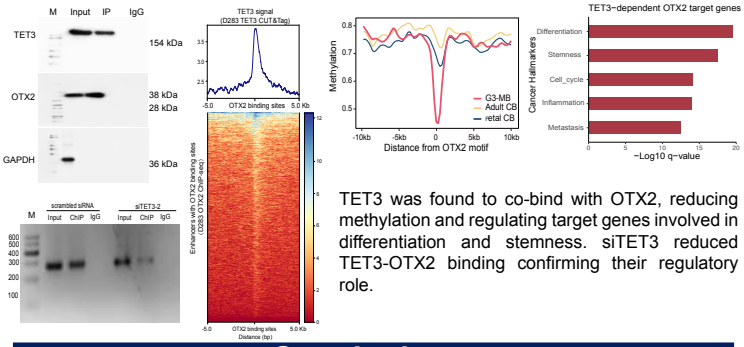
Results

I. Otx2 feed-forward autoregulates its own SE activity



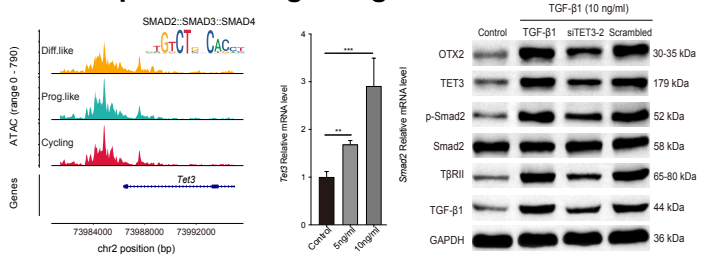
Schematic model showing the luciferase-reporter constructs of Otx2 enhancer mutant. Deletion of the OTX2 motif reduces cell proliferation and invasion ability.

II. OTX2-recruited TET3 demethylated the Otx2 SE



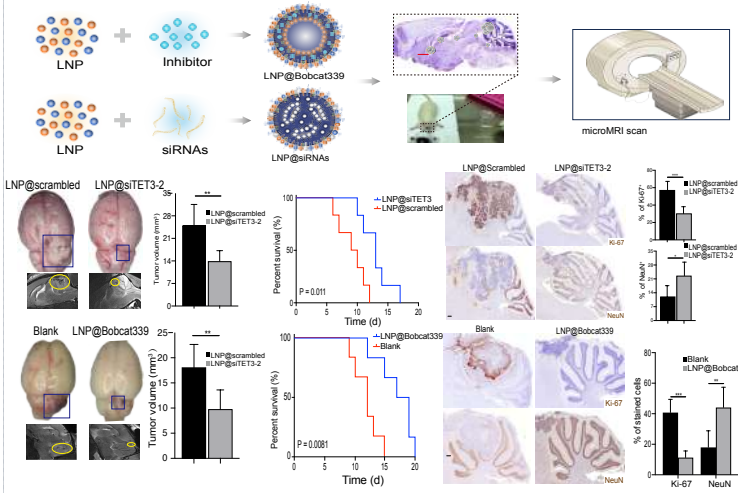
TET3 was found to co-bind with OTX2, reducing methylation and regulating target genes involved in differentiation and stemness. siTET3 reduced TET3-OTX2 binding confirming their regulatory role.

III. TGF-β1/Smad2 signaling activated the TET3



- Chromatin accessibility (CA) plots around the Tet3 promoter show Smads complex binding sites in three tumor cell populations in Group3 medulloblastoma.
- Treatment with TGF-β1 (5 ng/ml or 10 ng/ml) significantly increased Tet3 and Smad2 mRNA levels in D283 cells.
- Protein expression of TGF-β1, TβRII, Smad2, phosphorylated Smad2 (p-Smad2), TET3, and OTX2 was analyzed in D283 cells treated with TGF-β1 (10 ng/ml), comparing scrambled siRNA with siTET3-2 conditions.

IV. Downregulation of TET3 attenuated G3-MB progression.



The experiment used LNP-coated siTET3 and Bobcat339 to target medulloblastoma in PDX. Both treatments reduced tumor volume, extended survival, decreased Ki-67 (proliferation marker), and increased NeuN (neuronal differentiation marker), indicating inhibited tumor growth and enhanced differentiation.

Conclusions

Ten-Eleven translocation 3 (TET3), specifically recruited by OTX2 and triggered by TGF-β1/Smad2 signaling, demethylated Otx2 SE and open chromatins, to sustain tumor proliferation and stemness. LNPs-coated siTET3 or Bobcat339 significantly inhibited Group3 MB tumor growth.

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Reference

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In relation to this presentation, I declare that there are no conflicts of interest.