Lysine Demethylase 4B (KDM4B): A Novel Epigenetic Target in Atypical Teratoid/Rhabdoid Tumor (ATRT).

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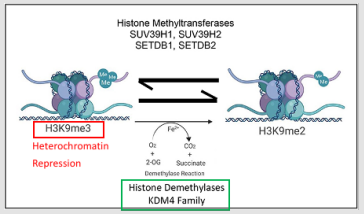
Introduction

Atypical teratoid/rhabdoid tumor (ATRT) is a highly aggressive childhood brain tumor; current treatment options are limited with intensive chemotherapy and radiation which often create therapy-related toxicity; this is especially critical in this young patient population. Previous studies reported the loss of SMARCB1, a member of ATP-dependent SWI/SNF chromatin remodeling complex, is the hallmark molecular feature of ATRT, creating an overall epigenetic dysregulation of ATRT genome. This marks a potential avenue in the search for targeted therapy. Here, we utilized an unbiased epigenome-wide RNAi screen and identified one of the top hits, histone lysine demethylase 4B (KDM4B), as a novel epigenetic regulator that is critical for ATRT growth.

Background: Atypical Teratoid/Rhabdoid Tumor (ATRT)

- Malignant central nervous system tumor in children
- 5-year survival of 35%
- Current therapy regiment: surgery, intensive chemo, radiation
- Salient molecular characterization: loss of SMARCB1 gene
  - Loss of function SWI/SNF chromatin complex and epigenetic dysregulation
- Different subgroups : TYR, SHH, MYC with various methylation patterns

Lysine demethylase 4B (KDM4B)



KDM4B ↑ H3K9Me3 ↑ Gene Expression
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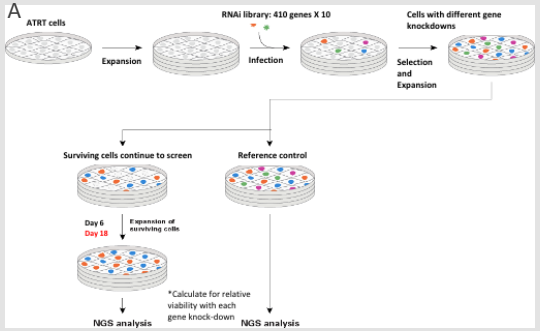
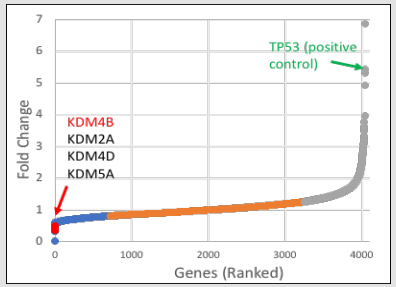
Study Questions

1) Examine KDM4B's (or KDM family genes) biological relevance in driving/maintaining the growth of ATRT cells
2) Determine mechanisms behind KDM4B function:
   -how does KDM4B loss alter histone markers, chromatin remodeling and transcription
3) Can we use KDM4B as a potential therapeutic target?

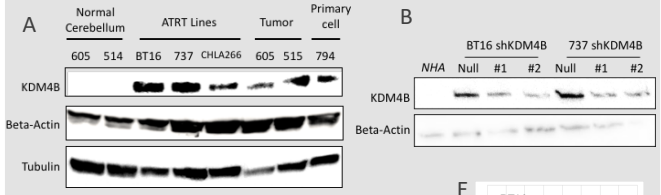
Epigenome wide RNAi screen

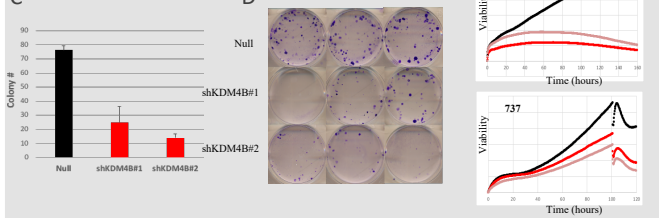


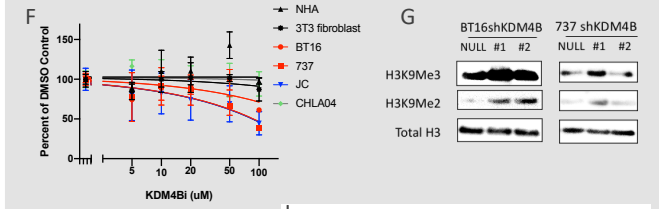


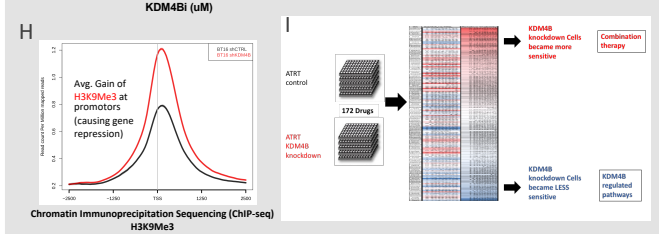
A. Design of the epigenome-wide shRNAi screen in the BT16 ATRT cell line. The pooled RNAi library contains ~4100 shRNAs corresponding to ~410 unique genes. Each shRNA contains sequencing barcode for identification. Cells were infected, selected, collected for control, and the rest passaged for 6 and 18 days. Cells were sequenced to compare changes in gene distribution after passage. viability is calculated using readout at Day 18 / Day 0. **B.** Scatter plot showing genes ranked by smallest relative viability to largest relative viability. Dots in blue are "hits" scoring <0.7 viability Day 18/ Day 0. KDM4B is among the top hits.

Results









A. Western blot showing baseline protein expression of KDM4B in ATRT cell lines (BT16, 737, CHLA606), primary cells (sample 794), snap frozen patient tumor samples (605, 515) vs. normal cerebellum patient samples (605, 514). **B.** western blot showing low throughput knockdown of KDM4B using dox-inducible shRNA, two cell lines were manipulated using two unique shKDM4B guides as biological replicates. **C.** Scatter plot showing viability of cells measured by xcelligence over 120 hours. shKDM4B knockdown #1, #2 (red), vs shNull control (black). **D.** colony formation assay using shKDM4B vs. shNull control cells. **E.** bar graph colony with formation number. **F.** pharmacologic inhibition of KDM4B using multiple ATRT cell lines (color) vs. Normal human Astrocytes (NHA) and fibroblast cells (black). **G.** western blot of histones showing upregulation of H3K9Me3 histone after shKDM4B knockdown. **H.** Chromatin Immunoprecipitation (ChIP) sequencing of H3K9Me3 showing global gain of peaks at promotor regions **I.** Drug screen with 132 FDA approved oncology drugs .

What we learned so far

1) *Examine KDM4B's (or KDM family genes) biological relevance in driving/maintaining the growth of ATRT cells*
- KDM4B loss engenders *decrease* in ATRT tumor cell viability
- KDM4B is *differentially* expressed at baseline in tumor cells and patient tumor samples vs control
  - Potential therapeutic window

2) *Determine mechanisms behind KDM4B function:*
- KDM4B loss leads to global upregulation of H3K9Me3 expression
  - More heterochromatin/global suppression of genome

3) *Can we use KDM4B as a potential therapeutic target?*
- Pharmacologic inhibition of KDM4B using small molecule tool compound differentially suppressed tumor cells without toxicity to normal human astrocytes and fibroblast
  - IC50 not ideal, need better chemical inhibitor

Next steps/Future Directions

- Integrated H3K9Me3 Chromatin immunoprecipitation (ChIP) sequencing and RNA sequencing shKDM4B knockdown vs control cells analysis
  - Identify pathways mediated by KDM4B
- KDM4B ChIP sequencing to explore its occupancy at promotors, enhancers and super enhancers in ATRT genome
  - Enhance our understanding in its role in ATRT genome
  - Elucidate role of KDM4B on chromatin remodeling
- Obtain novel KDM4B inhibitor and optimize for translational potential
- Test potential combination therapies
- Animal studies/preclinical testing

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