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

Emily Jue Wang¹, Etienne Danis², Irina Alimova³, Sujatha Venkataraman¹,², Rajeev Vibhakar¹,²

Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA¹
Morgan Adams Foundation Pediatric Brain Tumor Research Program, Aurora, CO, USA²

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 complex, is the hallmark molecular feature of ATRT, creating an overall epigenetic dysregulation of ATRT genome. This marks a complex relationship between ATRT tumor cells and patient tumor samples vs control 

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What we learned so far

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?

Study Questions

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Epigenome wide RNAi screen

Design of the epigenome-wide RNAi screen in the BT16 ATRT cell line. The pooled shRNA 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.

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 human astrocytes and fibroblast. Pharmacologic inhibition of KDM4B using dox-inducible shRNA suppression of H3K9Me3 histone after shKDM4B knockdown. B. Chromatin immunoprecipitation sequencing (ChIP-seq) of H3K9Me3 showing global gain of peaks at promotors, enhancers and super enhancers in ATRT genome. C. Scatter plot showing viability of cells measured by xcelligence treatment KCN4B vs. control (black). D. Western blot showing KDM4B loss engenders decrease in ATRT tumor cell viability. E. Potential therapeutic window. F. Dose response to pharmacologic inhibition of KDM4B showing global gain of peaks at promotors, enhancers and super enhancers in ATRT genome. G. Potential therapeutic window. H. Western blot showing inhibition of KDM4B using multiple ATRT cell lines (BT16, 737, CHLA606) primary cells (sample 794), snap frozen patient tumor samples (605, 515) vs. normal human astrocytes and fibroblast.

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