Mechanisms of ETS1/KDM3A Interaction with PAX3-FOXO1 in Fusion-Positive Rhabdomyosarcoma

Joseph (Joe) Hsieh, Lays Sobral, and Paul Jedlicka

University of Colorado Anschutz Medical Campus

Cancer Biology Program; Medical Scientist Training Program; Departments of Pathology and Pediatrics

Introduction

Rhabdomyosarcoma (RMS), a malignancy of mesenchymal origin with impaired myogenic differentiation, is the most common pediatric soft tissue cancer. Molecularly, RMS is classified into fusion-negative and **fusion-positive RMS (FP-RMS)**. Clinically, the fusion status has strong prognostic value with FP-RMS representing a highly aggressive disease with dismal outcomes. Thus, there is a dire need for increased knowledge of FP-RMS oncogenesis and therapeutic opportunities.

The fusion forms a novel oncogenic pioneer factor involving PAX3 and FOXO1 that drives aberrant gene expression, promotes metastasis, and impairs differentiation. The oncofusion **PAX3-FOXO1 (P3F)** employs epigenetic mechanisms, such as **activation of enhancers and recruitment of chromatin factors**, to drive FP-RMS disease gene expression. Given the difficulties in targeting P3F, understanding of P3F cofactors may reveal alternative therapeutic strategies for FP-RMS.

Preliminary Data

A P3F/KDM3A/ETS1 regulatory axis driving FP-RMS

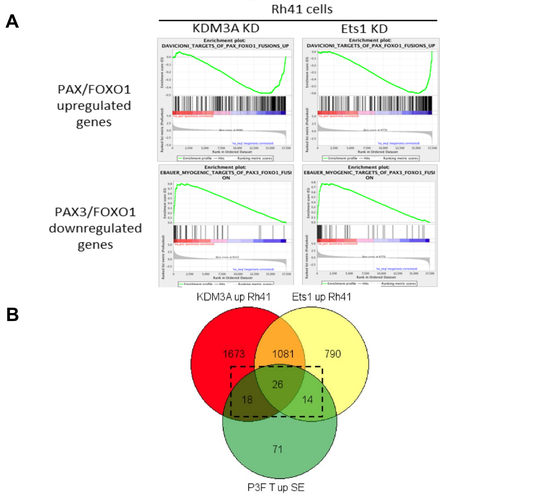


Fig. 1: P3F/KDM3A/ETS1 regulatory axis.
A: GSEA shows down-regulation of P3F-activated genes and up-regulation of P3F-repressed myogenic differentiation genes upon knockdown of KDM3A or ETS1.
B: Venn diagram showing overlap of distinct set of genes under KDM3A/ETS1/P3F regulation that are associated with SEs.
C: Example candidate genes directly bound by P3F, associated with SEs, and under KDM3A/ETS1 regulation, including notably PODXL.

Results

ETS1 and KDM3A colocalize with P3F, as well as BRG1, at FP-RMS disease-gene enhancers.

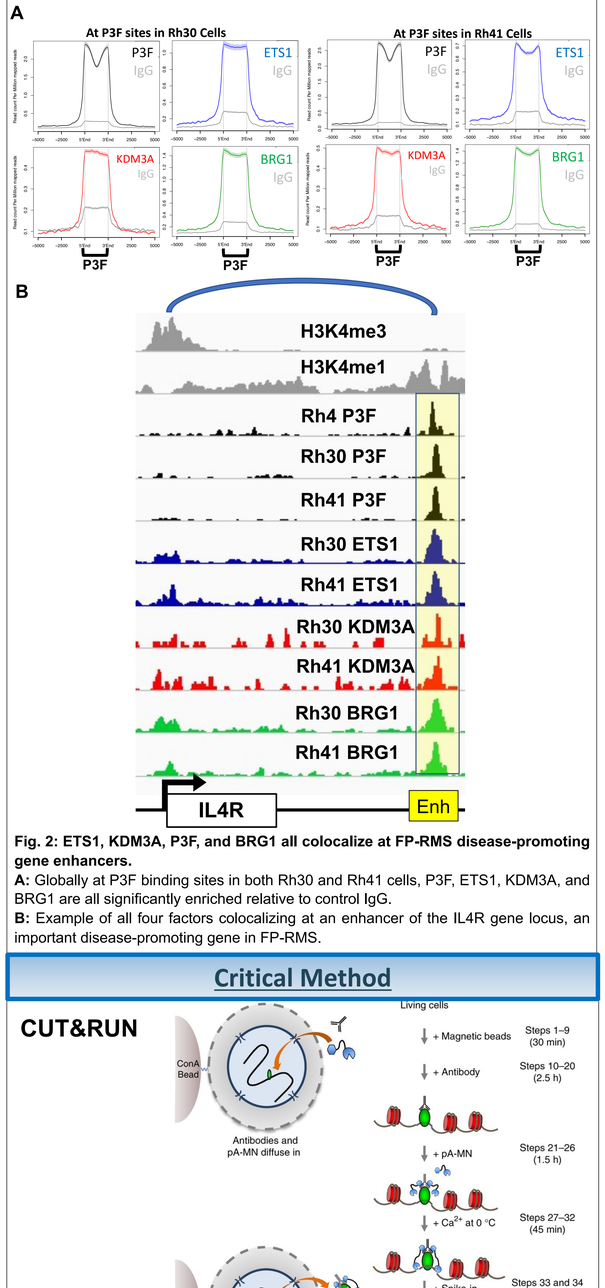


Fig. 2: ETS1, KDM3A, P3F, and BRG1 all colocalize at FP-RMS disease-promoting gene enhancers.
A: Globally at P3F binding sites in both Rh30 and Rh41 cells, P3F, ETS1, KDM3A, and BRG1 are all significantly enriched relative to control IgG.
B: Example of all four factors colocalizing at an enhancer of the IL4R gene locus, an important disease-promoting gene in FP-RMS.

Critical Method

Fig. 5: Schematic of CUT&RUN genome profiling methodology.
Schematic figure from Skene & Henikoff, 2017.

ETS1 and BRG1 regulate each other's, and KDM3A and P3F chromatin recruitment.

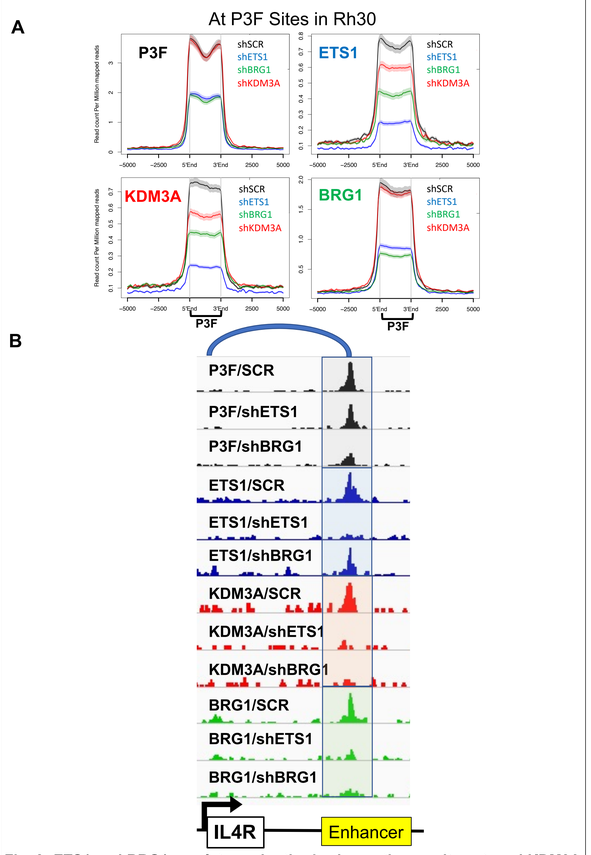
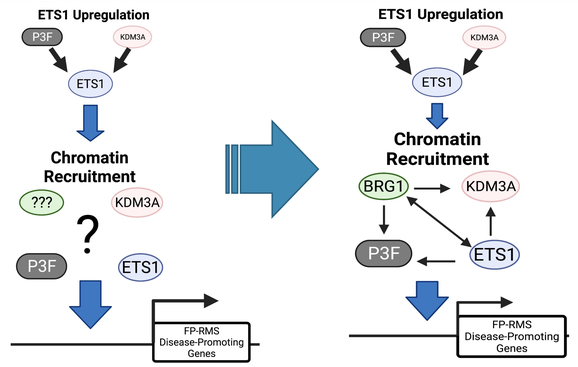


Fig. 3: ETS1 and BRG1 regulate each other's chromatin recruitment, and KDM3A and P3F chromatin localization as well.
A: Enrichment of each factor globally at P3F binding sites in Rh30 cells upon different factor knockdown relative to scrambled control.
B: Example cistrome at the IL4R locus showing chromatin recruitment changes upon either ETS1 or BRG1 knockdown.

How do P3F, KDM3A, ETS1, and cofactors together regulate expression of FP-RMS disease-promoting genes?.



Gene expression changes only subtly influenced by H3K27ac changes.

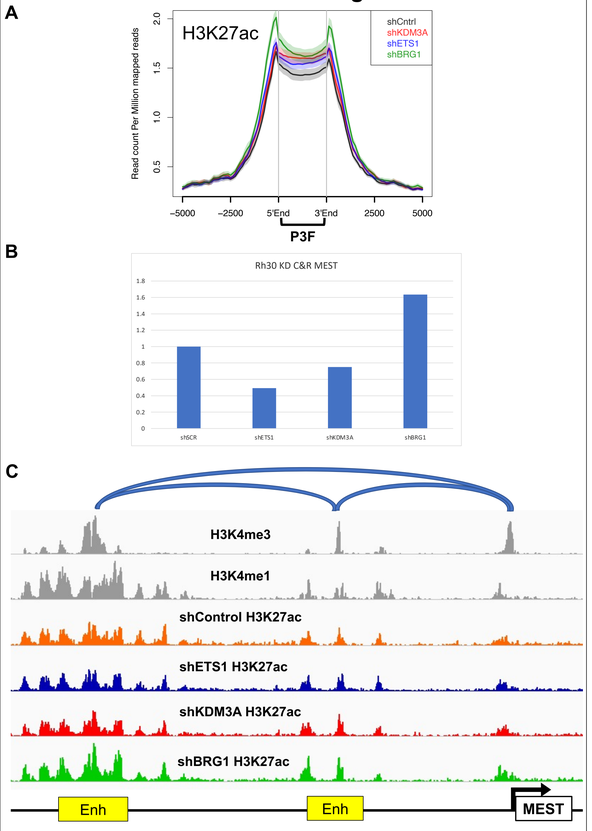


Fig. 4: Gene expression changes are only subtly influenced by H3K27ac.
A: Levels of H3K27ac upon factor knockdown globally at P3F binding sites.
B: MEST expression changes upon each factor knockdown confirmed by qRT-PCR.
C: Cistrome at the MEST locus showing subtle changes in H3K27ac levels upon each factor knockdown.

Conclusions

- ETS1, KDM3A, P3F, and BRG1 colocalize at disease-promoting gene enhancers.
- ETS1 and BRG1 regulate each other's localization to disease gene loci, and regulate KDM3A and P3F chromatin binding.
- Gene expression changes only minimally influenced by H3K27ac levels.

Future Directions

- Verify chromatin recruitment findings in Rh41 cells and examine P3F role in chromatin localization of cofactors.
- Role of ETS1 in modulating P3F chromatin remodeling pioneer activity.
- Role of pETS1-T38 in modulating chromatin recruitment and disease phenotypes.
- Protein-protein interactions between factors.