THE CHROMATIN REMODELING PROTEIN ARID1A IS REQUIRED FOR MLL-AF9 LEUKEMOGENESIS

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INTRODUCTION

The SWI/SNF chromatin remodeling complexes (BAF, PBAF, and ncBAF) regulate gene transcription by modulating chromatin accessibility. They share core subunits and are distinguished by unique subunits including the ARID proteins: ARID1A /1B (BAF) and ARID2 (PBAF). Despite SWI/SNF complexes' prominent role as tumor suppressors in solid tumors, previous studies have shown that they are mostly required for AML leukemogenesis. Clinical study of small molecule inhibition of the common ATPase subunits for AML is impeded by dose limiting toxicities, which raises the question whether targeting of specific subcomplexes would be preferred. The functions of specific subcomplexes in normal and malignant hematopoiesis are still being understood. We previously showed that loss of ARID2 and ARID1B have opposing roles in AML leukemogenesis: ARID2-loss impairs while ARID1B loss promotes leukemogenesis[1], highlighting the importance of understanding the role of different BAF subcomplexes. In this study, we characterize the role of ARID1A in AML leukemogenesis using patient data, mouse models and subcomplex-specific inhibitors.

METHODS

Patient data

Tempus Lens database was used to identify AML patients (excluding acute promyelocytic leukemia (APL)) with both RNA-seq and DNA-seq data (n=592). RNA-seq data were normalized for batch effects, quantified as transcripts per million (TPM) and reported as log2(TPM+1). Based on quartiled *ARID1A* expression, samples were grouped as *ARID1A low* (n=150) or *ARID1A high* (n=148). Cox proportional hazards models assessed real-world overall survival (from diagnosis to death) and mutational profiles were compared between groups.

In vitro analysis

Retroviral transformation was used to generate leukemic model cells from wildtype and *Arid1a*$^{f/f}$ mouse cells. Upon CRE-induced deletion, BrdU incorporation was used to assess proliferation, and colony forming cell assays were used to assess clonogenic ability. Levels of maturation markers for myeloid lineages were measured by flow cytometry, as were levels of apoptosis by Annexin V staining. Similar measurements of differentiation and apoptosis were performed in human and mouse AML cell lines using both SWI/SNF subcomplex-specific and nonspecific inhibitors.

RESULTS

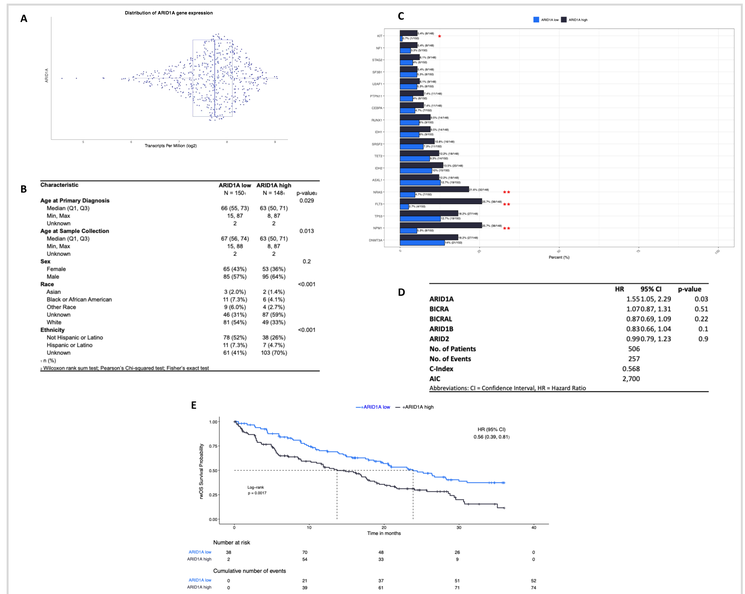

Figure 1. ARID1A (A) Distribution of ARID1A expression was quantified as transcripts per million using RNA-seq data obtained from 592 unique patients with AML with both Tempus xT (DNA-seq) and xR (RNA-seq) data, excluding acute promyelocytic leukemia. For patients where multiple samples were sequenced, one sample closest to the primary diagnosis was chosen for analysis. **(B)** Patient samples were classified as ARID1A-low and ARID1A-high based on top and bottom quartiles of gene expression. Table displays demographic characteristics of these two expression quartiles. **(C)** Profile of co-mutations in genes subject to recurrent mutations in AML for ARID1A-low vs. ARID1A-high cases. *p-value 0.019 **p-value <0.001. Not shown is KMT2A, mutations in which are also enriched in ARID1A-high cases (p=0.014). **(D)** Multivariate Cox proportional hazard regression was performed for gene expression as continuous variable, accounting for other BAF complex genes (BICRA, BICRAL, ARID1B, ARID2). Only increased ARID1A expression was significantly associated with worse survival. **(E)** Kaplan-Meier estimation of real-world overall survival (rwOS) probability was performed for ARID1A_low and ARID1A_low patients. The index date is the date of diagnosis, and the entry date is the date of sequencing. Low ARID1A expression was significantly associated with improved rwOS (HR = 0.56; 95% CI: 0.39 - 0.81; p=0.002).

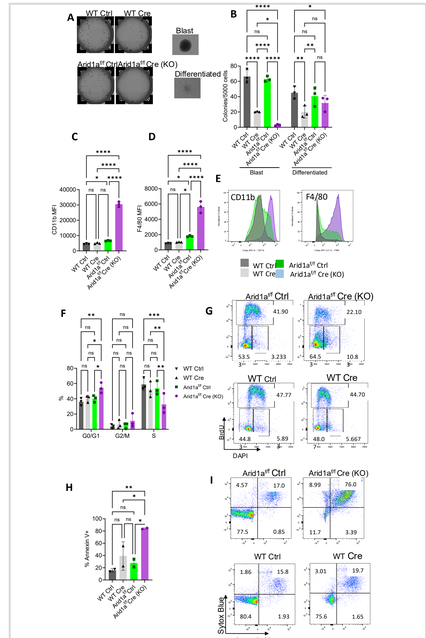

Figure 2. Effect of ARID1A loss in mouse MLL-AF9 leukemia cells. (A-B) CFC of preleukemic murine MLL-AF9 cells after transduction of CRE or MIT control viruses in methylcellulose. Results of technical triplicates. Plate images in A and colony counts in B. **(C-E)** Differentiation mark analysis by flow cytometry. C-D show MFI (geometric mean fluorescent intensity) plots and E histograms. Results from three independently transduced triplicate samples. **(F-G)** Plots of cell cycle analysis and flow cytometry representative plots following BrdU staining. Results from three independently transduced triplicates. **(H-I)** Plots of apoptosis analysis and flow cytometry representative plots. Results from two independent experiments. Unpaired two-sided student t-test *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

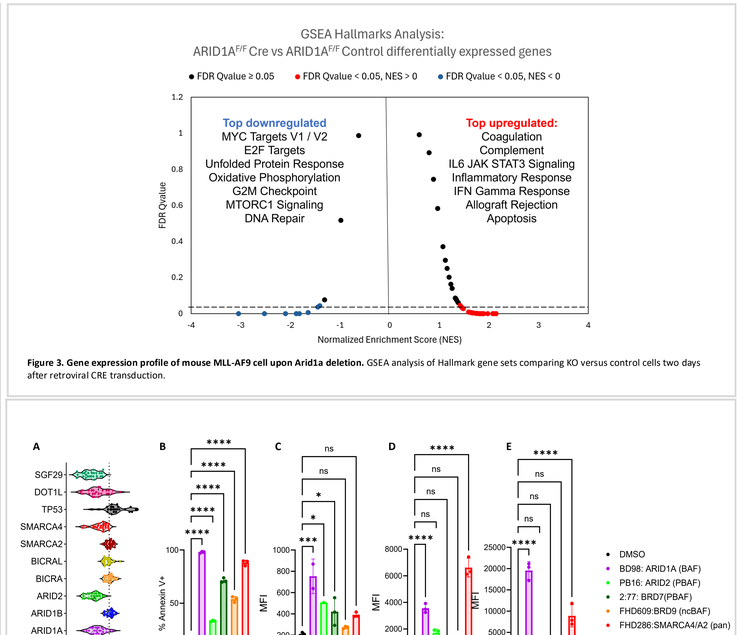

Figure 3. Gene expression profile of mouse MLL-AF9 cell upon Arid1a deletion. GSEA analysis of Hallmark gene sets comparing KO versus control cells two days after retroviral CRE transduction.

Figure 4. SWI/SNF subunits inhibition in AML cell lines. (A) Depmap data from 26 AML cell lines shows reduced fitness upon knockout of ARID1A. . Negative gene effect score indicates reduces fitness upon knockout of the gene (DOT1L and SGF29 as examples) and positive score indicates increased fitness upon gene knockout (e.g P53) **(B-E)** Flow cytometric analysis of effect of SWI/SNF complex inhibitors on apoptosis and cell differentiation in CD34 MLL-AF9 cells. Results from three independently treated triplicates samples.

CONCLUSIONS

- Enrichment of oncogenic mutations (KIT, NRAS, FLT3, NPM1) in AML patients with high ARID1A expression and significant association of low ARID1A expression with improved survival indicate high ARID1A expression may identify a higher risk disease state compared to low ARID1A expression.
- Deletion of ARID1A in murine retroviral model of MLL-AF9 AML leads to decreased proliferation, increased cell differentiation/death, and impaired clonogenic ability, suggesting that ARID1A is essential for AML cell survival.
- Phenotypes observed in human leukemia cell lines treated with BAF subcomplex-specific inhibitors indicate that ARID1A-specific inhibition may achieve similar efficacy as ATPase inhibition.
- Studies are ongoing to define the effect of ARID1A-mediated leukemogenesis through transcriptomic and epigenomic studies and in vivo models.

REFERENCES

[1]**Bleumn T., et al**. Differential roles of BAF and PBAF subunits, Arid1b and Arid2, in MLL-AF9 leukemogenesis. *Leukemia 2022; 36(4):946-955*

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