ARID1A prevents H3K27-hyperacetylation at active super-enhancers

Jake J. Reske[1], Mike R. Wilson[1], Ronald L. Chandler[1,2,*].

[1]Department of Obstetrics, Gynecology and Reproductive Biology, College of Human Medicine, Michigan State University, Grand Rapids, MI, USA

[2]Center for Epigenetics, Van Andel Research Institute, Grand Rapids, MI, USA

@reskejak

email: reskejak@msu.edu

*email: rlc@msu.edu

ABSTRACT

Mutations in the multi-subunit SWI/SNF (BAF) chromatin remodeling complex are observed in nearly all types of cancer. The most highly mutated subunit across cancer is ARID1A, which occurs in roughly half of uterine endometrial cancer cases. ARID1A mutations and loss of expression are also common in endometriosis. We have previously demonstrated that ARID1A loss drives transcriptional alterations leading to invasive phenotypes through multiple model systems. Yet, how ARID1A regulates transcription through chromatin regulation is poorly understood. We have generated genome-wide profiles of seven chromatin features associated with transcriptional regulation in human endometrial epithelial cells, in both the presence and absence of ARID1A, in order to identify a mechanism by which ARID1A regulates chromatin. Unbiased genome segmentation using these features revealed that ARID1A interacts with chromatin at highly active and accessible enhancers genome-wide. Out of all tested chromatin features, ARID1A most strongly co-localizes with ATAC (accessibility) and H3K27ac, a mark of transcriptional activation at proximal and distal enhancers. ARID1A depletion also displayed the greatest effect on genomic H3K27ac as opposed to other histone modifications, H3K18ac, H3K4me1, H3K4me3, and H3K27me3. While the majority of typical enhancers had reduced H3K27ac with ARID1A loss, super-enhancers marked by high basal H3K27ac levels displayed hyperacetylation following ARID1A loss. Moreover, increasing H3K27ac was also associated with heightened eRNA transcription, indicating enhancer hyperactivation. Finally, RNA-seq revealed that genes associated with active super-enhancers were preferentially dysregulated following ARID1A loss. These results suggest that ARID1A-SWI/SNF differentially regulate typical enhancers vs. super-enhancers in the endometrial epithelium, and ARID1A may normally restrict super-enhancer hyperactivation to maintain epithelial identity and prevent endometrial invasion.

Building a genome-wide chromatin state map accompanying ARID1A loss in human endometrial epithelial cells

Profiled chromatin features:

- **Total RNA:** transcriptional activity
- **ATAC**: chromatin accessibility
- **H3K27ac**: active regulatory elements; enhancers
- **H3K18ac**: (active) regulatory elements; enhancers
- **H3K4me1**: both poised and active regulatory elements / enhancers
- **H3K4me3**: active gene promoters and TSS (incl. some highly active enhancers)
- **H3K27me3**: polycomb-mediated repressed genomic elements

All features profiled in both wild-type (control) and ARID1A-knockdown cells

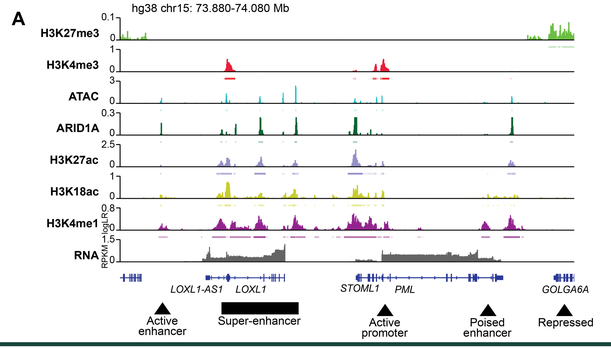

Fig. 1. (**A**) Example hg38 locus displaying profiled chromatin features in control cells, including ARID1A binding by ChIP-seq. Aside from RNA, *y*-axis is signal displayed as log-likelihood ratio (logLR) as reported by *MACS2*. Significant peaks (FDR < 0.05) are demarcated by small bars underneath signal tracks. (**B**) 18-state *chromHMM* model built from the seven chromatin features in both control and ARID1A-deficient cells. (**C**) Genomic enrichment for ARID1A binding at each chromatin state as compared to the whole genome. Statistic is hypergeometric enrichment.

Identifying and distinguishing active super-enhancers and typical enhancers

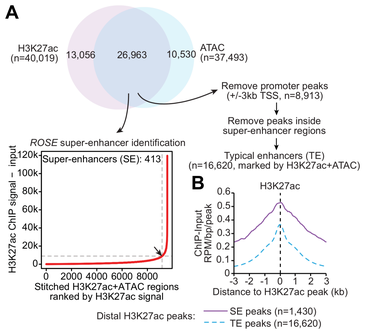

Fig. 2. (**A**) Diagram depicting enhancer identification and segregation workflow. Genome-wide H3K27ac peaks were first filtered for only those co-marked by ATAC (accessibility). ATAC+ H3K27ac peaks were then subject to *ROSE* ranking. The remaining non-super-enhancer peaks were then filtered to remove promoter peaks and finally designated as typical enhancers. (**B**) Histogram of H3K27ac signal at distal super-enhancer (SE) vs. typical enhancer (TE) constituent peaks.

ARID1A is strongly associated with active regulatory elements marked by ATAC and H3K27ac

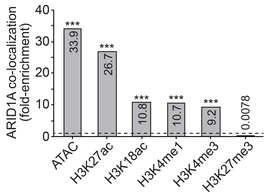

Fig. 3. Genome-wide chromatin feature association with ARID1A binding. Co-localization was quantified by fold-enrichment at ARID1A-bound vs. not bound genomic base pairs (bp), genome-wide.

Loss of ARID1A does not elicit substantial epigenomic reprogramming

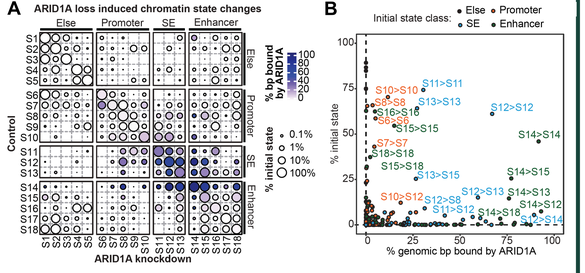

Fig. 4. (**A**) 18 x 18 chromatin state-state transition map following ARID1A loss. Each cell [*i*,*j*] represents genomic bp transitioning from state *i* in control cells to state *j* in ARID1A-knockdown cells. Larger circles indicate a greater state representation, and darker colors indicate greater ARID1A binding association. (**B**) Scatter of all 324 possible state-state transitions quantified by features as in **A**.

ARID1A loss disrupts genomic H3K27ac regulation associated with enhancer activation state dynamics

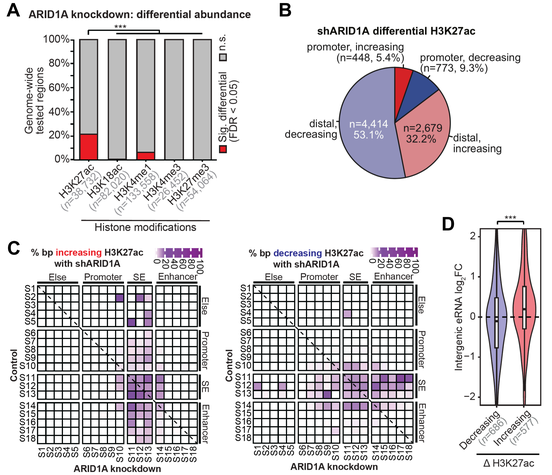

Fig. 5. (**A**) Proportion of tested genomic regions displaying significant differential abundance (FDR < 0.05) following ARID1A knockdown for each profiled post-translational histone modification. Statistic is two-tailed Fisher's exact test. (**B**) Distribution of differential H3K27ac regions genome-wide, segregated by increasing vs. decreasing acetylation and promoter proximal (<3 kb from TSS) vs. distal (>3 kb from TSS). (**C**) 18 x 18 chromatin state-state transition map as in Fig. 4A but displaying (left) proportion of bp displaying increasing H3K27ac following ARID1A loss vs. (right) decreasing H3K27ac. (**D**) Enhancer RNA (eRNA) expression changes following ARID1A loss at intergenic regions displaying increasing vs. decreasing H3K27ac. Statistic is unpaired, two-tailed Wilcoxon test.

ARID1A differentially regulates super-enhancers vs. typical enhancers

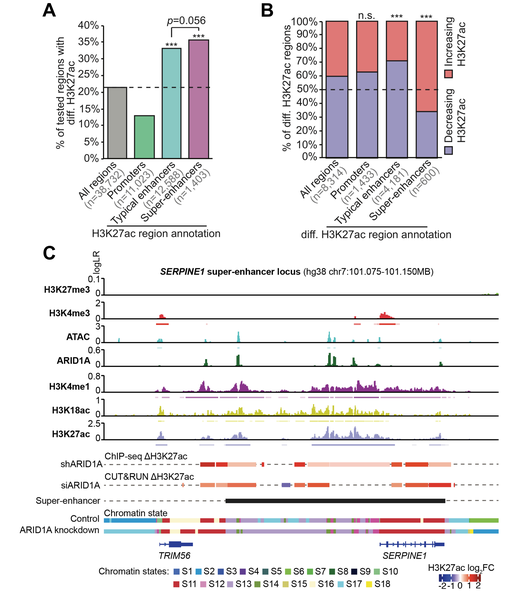

Fig. 6. (**A**) Enrichment of differential H3K27ac sites at promoters vs. SE vs. TE. (**B**) Distribution of increasing vs. decreasing H3K27ac at promoters vs. SE vs. TE. (**C**) Example locus depicting super-enhancer hyperacetylation following ARID1A loss.