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Original Article

Down-regulation of ARID1A is sufficient to initiate neoplastic transformation along with epigenetic reprogramming in non-tumorigenic endometriotic cells

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ABSTRACT

The chromatin remodeler AT-Rich Interactive Domain 1A (ARID1A) is frequently mutated in ovarian clear cell carcinoma (OCCC) and endometriosis precursor lesions. Here, we show that knocking down ARID1A in an immortalized endometriosis cell line is sufficient to induce phenotypic changes indicative of neoplastic transformation as evidenced by higher efficiency of anchorage-independent growth, increased propensity to adhere to collagen, and greater capacity to invade basement membrane extract *in vitro*. ARID1A knockdown is associated with expression dysregulation of 99 target genes, and many of these expression changes are also observed in primary OCCC tissues. Further, pathway analysis indicates these genes fall within networks highly relevant to tumorigenesis including integrin and paxillin pathways. We demonstrate that the down-regulation of ARID1A does not markedly alter global chromatin accessibility or DNA methylation but unexpectedly, we find strong increases in the active H3K27ac mark in promoter regions and decreases of H3K27ac at potential enhancers. Taken together, these data provide evidence that ARID1A mutation can be an early stage event in the oncogenic transformation of endometriosis cells giving rise to OCCC.

Introduction

Epithelial ovarian cancer (EOC) has the highest mortality of all female reproductive malignancies in the United States [1]. Ovarian clear cell carcinoma (OCCC) accounts for 5–25% of EOCs and the advanced stages of OCCC have the worst patient outcomes of all EOC subtypes [2–4]. Lack of effective therapies and particularly resistance to conventional platinum-based chemotherapy contribute to the poor prognosis of late-stage OCCC [5,6]. It is thought that OCCC arises from atypical endometriosis, however, a causal relationship between endometriosis and OCCC remains to be established [7,8]. Thus, better understanding of the biological and functional processes governing the pathogenesis of OCCC is crucial to the development of more effective early detection tools and efficient treatment strategies.

Several studies have found that the SWI/SNF chromatin remodeler member AT-Rich Interactive Domain 1A (ARID1A) is mutated in numerous cancer types and is the most commonly mutated gene in OCCC (46%–57%) and endometrioid carcinoma (26%–40%) [9–12]. OCCC shows the strongest association of any

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ovarian subtype with pre-existing endometriosis [7,10,13], and notably, mutation or loss of ARID1A expression has been noted in 15%–44% of endometriotic lesions [10,14,15]. Consequently, it has been proposed that ARID1A mutation might be an early event in the oncogenic transformation of endometriotic cells to OCCC [7,12,16].

Here, we investigate the potential functional role and the epigenetic impact of decreased ARID1A expression on oncogenesis by using an immortalized endometriosis cell line model, iEEC16 [17,18], to evaluate for alterations of DNA methylation, nucleosome positioning, and histone modifications following ARID1A perturbation. Our studies reveal that the down-regulation of ARID1A expression in non-tumorigenic cells is sufficient to initiate tumorigenic transformation and dysregulation of gene expression through altered H3K27ac and targeted modulation of chromatin accessibility.

Materials and methods

Cell lines and reagents

IEEC16 was derived in-house from primary endometriosis sample and cultured in normal ovarian surface epithelial complete medium [17,18]. Cell lines were characterized by STR-profiling and tested for mycoplasma at USC Norris Cancer Center. Lentiviral vector pLKO.1 with short-hairpin RNAs (shRNAs) targeting human *ARID1A* (clone IDs: TRCN0000059089, and TRCN0000059090) or a non-targeting scrambled sequence (Dharmacon) and packaging plasmids pCML1 and pMDG1 were used with Lipofectamine LTX Reagent with Plus (ThermoFisher) to generate virus. Cells were selected with 1 μg/ml puromycin.

Protein extraction and western blot analysis

Western blot was performed as described in [19]. Antibodies against ARID1A (Santa Cruz Biotechnology, sc-32761, 1:500, Dallas, TX) and β-ACTIN (Sigma, A2228, 1:2000) were used.

ARID1A-KD and SCR in-vitro characterization

Anchorage-independent colony formation and cell adhesion assays were performed as described in [18,20]. Cell invasion assay performed using Cultrex® BME cell invasion assay (R&D Systems) according to manufacturer's protocol.

NOMe-seq assay

Nucleosome-occupancy and DNA methylome sequencing (NOMe-seq) and data analyses were performed as previously described in [21,22]. For locus-specific analysis, nuclei were treated with GpC Methyltranferase (NEB), genomic DNA was extracted and bisulfite-converted using EZ-DNA methylation kit (Zymo) and region specific primers (listed in Supplemental Table 1) were used to PCR-amplify the product which were then cloned and sequenced.

RNA extraction and analysis

Expression microarray analysis was performed and processed as previously described in Ref. [23] using BeadChipHumanHT12_V4 (Illumina). Pathway analysis was performed using Ingenuity Pathway Analysis (IPA, Qiagen). Oncomine™ (https://www.oncomine.org/) was used to mine publically available gene expression data (Qiagen). Quantitative PCR (qPCR) analysis was performed as described in Ref. [19]. Primers used in qPCR analysis are listed in Supplemental Table 1.

Chromatin immunoprecipitation sequencing (ChIP-seq), peak-calling, peak-annotation

ChIP-seq was performed as previously described [22]. ChIP assays were performed in replicates with scrambled control (SCR) cells; ChIP-assays in ARID1A-knockdown 1 and ARID1A-knockdown 2 samples were treated as biological replicates. The following antibodies from ActiveMotif were used: H3K4me3 (39160), H3K4me1 (39298), H3K27ac (39297), H3K27me3 (39155).

Unique ChIP-seq reads were mapped to hg19 using SeqMonk (http://www.bioinformatics.babraham.ac.uk/projects/seqmonk). Peaks were called on biological replicates using the model-based analysis of ChIP-seq (MACS) [24] algorithm with a p-value cutoff of 10^{-10} against input and only peaks common among the replicates were retained. Read count quantification, correcting for data store size and peak width, followed by log2 transformation was used to quantify peaks.

Active promoters were identified by the presence of H3K27ac and H3K4me3 within 2 kb of an annotated TSS, inactive promoters were identified by H3K27me3 peaks overlapping TSS and H3K27ac peaks in regions >2 kb away from TSS marked by the presence of H3K4me1 and absence of H3K4me3 were designated as enhancers [25–27]. A fold-difference of 1.5 between peak values in SCR and KD cells was used as a cut-off of change. Promoter peaks were annotated with overlapping gene. Enhancers often regulate multiple genes over considerable distances, however, studies have shown that enhancers affect the gene corresponding to the nearest TSS with a higher frequency than any other TSS in the genome [28,29]. Therefore, putative enhancer peaks were annotated with the nearest gene within 200 kb of the peak.

Data analysis and statistics

Data analysis and visualization was performed using the statistical language R. NOMe-seq data analyses were performed as detailed in [22]. Two-tailed Student's t-test was performed and a p-value cut-off of 0.05 used for all statistical analysis unless mentioned otherwise. Bonferroni correction was applied when appropriate [30]. All data shown represent results of triplicated experiments (mean ± S.E.). The datasets supporting the results of this article are available at Gene Expression Omnibus (GEO) repository: GSE86572, GSE86810, GSE97373.

Results

Decreased ARID1A expression in an endometriosis cell line enhances colony formation capacity, cell adhesiveness, and invasiveness

To test whether ARID1A deficiency could be an early event in the transformation of endometriotic lesions to OCCC, we established stable knockdowns in the immortalized endometriosis cell line iEEC16. The down-regulation of ARID1A was confirmed at the mRNA and protein levels with independent shRNAs (KD1 and KD2) (Fig. 1a). Endometriosis cells transduced with shRNA to knockdown *ARID1A* gene expression showed a statistically significant increase in anchorage-independent colony formation in soft agar relative to cells transduced with a non-targeting scrambled control (SCR) shRNA (p-value < 0.05) (Fig. 1b). In addition, ARID1A knockdown cells displayed a tendency towards increased adhesion to collagen I and greater invasion through basement membrane extract (p-value < 0.05) (Fig. 1c, and d). Thus, our cell line model supports our hypothesis that loss of ARID1A can instigate the acquisition of phenotypes associated with oncogenic transformation of endometriotic lesions.

Dysregulation of gene expression in ARID1A knockdowns mimic the expression changes in OCCC

To understand how reduction in ARID1A expression could contribute to direct functional changes in the progression from endometriosis to OCCC, we performed gene expression microarray analysis of the SCR and KD cell lines. We observed 99 genes with significant expression changes in both KD lines relative to the control (Fold Change (KD/SCR) > 1.4, p-value < 0.05) (Fig. 2a, Supplemental Table 2). Comparing these expression data with publicly available data on primary OCCC tumors obtained using the Oncomine™ data portal, we observed a similar expression pattern in primary OCCC tissues for many of the genes dysregulated in our ARID1A KDs. These include gain of *GRPr* expression and loss of *SULF1* expression. *GRPr*, often upregulated in several solid tumor types, behaves as a growth factor contributing to tumor proliferation [31]. *SULF1*, frequently down-regulated in ovarian clear cell cancer, is known to promote cellular proliferation through growth factor signaling [32]. The top 20 genes with observed differential expression changes correlating with expression data from primary OCCC are summarized in Fig. 2b.

Pathway analysis of the genes differentially expressed in the iEEC16 KD cell lines revealed that networks most significantly altered (p-value < 0.05) were either related to cancer or immune response (Fig. 2c, Supplemental Fig. 1a). Notable affected pathways included the integrin signaling pathway, which contributes to several cellular functions including cell adhesion and invasion, and the paxillin pathway, which is critical in mediating cell integrin signaling with p38, MAPK and JNK pathways, thereby altering cell proliferation and motility [33,34]. Thus, gene expression alterations

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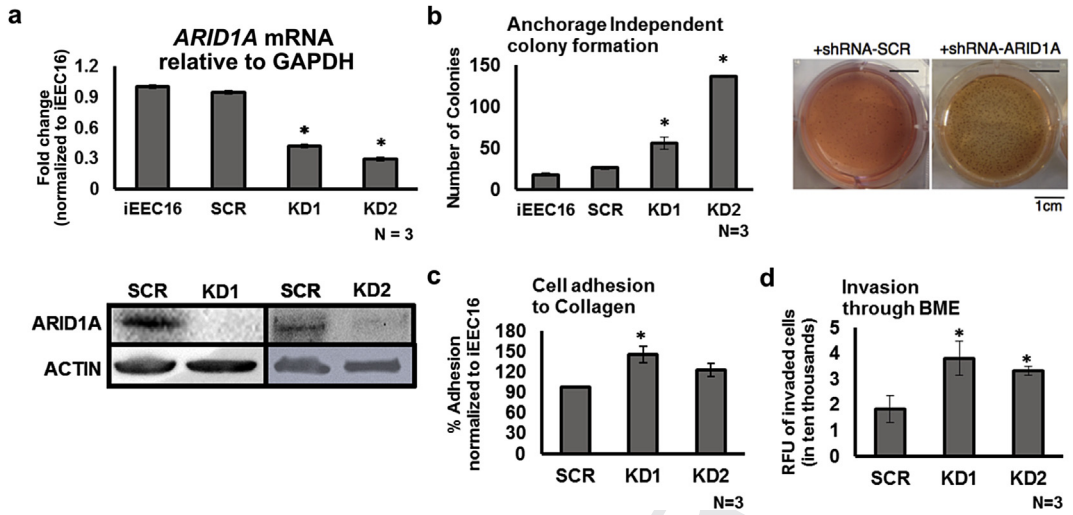

Fig. 1. ARID1A KD lines established in iEEC16 show phenotypic alterations. Two ARID1A knockdown (KD1 and KD2) and one scrambled control (SCR) lines were established in iEEC16. (a) Bar-graph of *ARID1A* mRNA levels (n = 3, p-value < 0.05) along with representative western blot image of ARID1A protein and beta-Actin loading control, bar-graph quantifying (b) the number of colonies grown in soft agar along with representative phase-contrast microscopy images, (c) cell adhesion to collagen measurement (n = 3, p-value < 0.05), and (d) fluorescence measurement of cells that successfully invaded through BME *in-vitro* (n = 3, p-value < 0.05).

in these pathways might be responsible for the increased cell adhesion to collagen and invasion observed in ARID1A-KD cell lines (Fig. 1c and d). Additionally, we identified genes that have been implicated in several gynecological malignancies including endometrial and ovarian cancer (Supplemental Fig. 1b). Finally, many of the upstream-regulators of the differentially expressed genes, including *Cg*, *TNF*, *PDGF-BB*, *VEGF*, *and RASSF1*, have been previously linked to ovarian cancer [35–38] (Supplemental Fig. 1c).

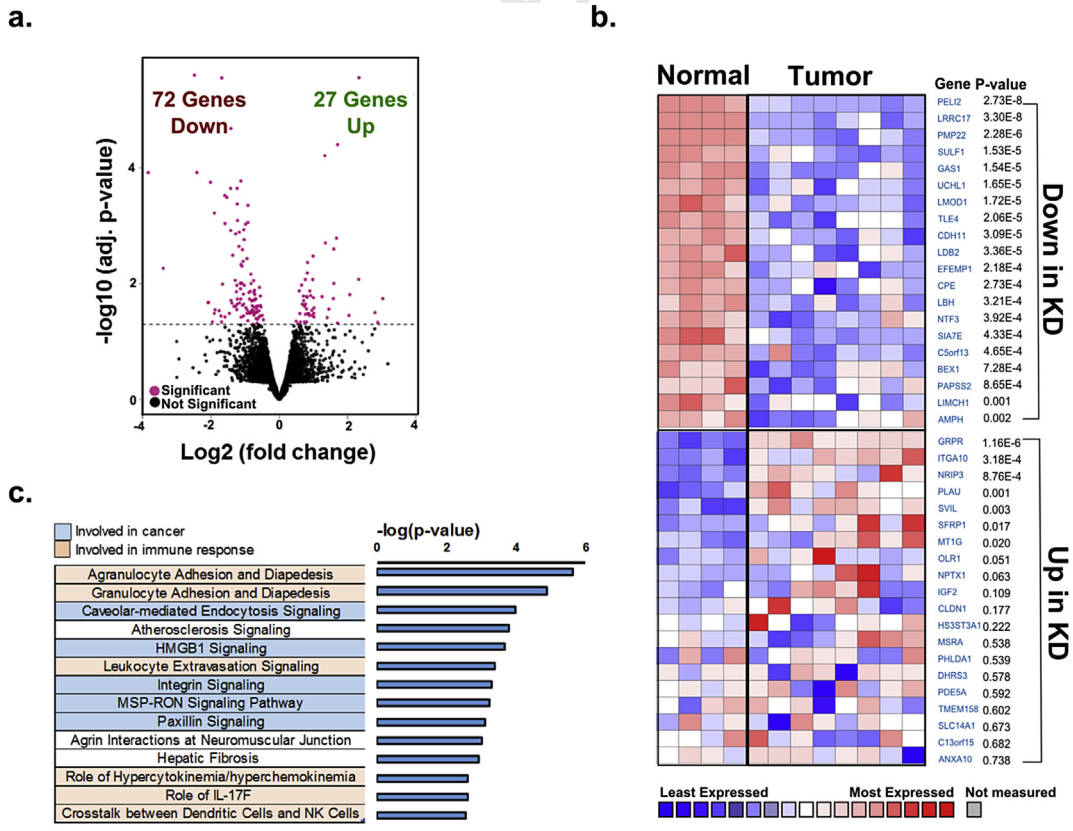

Fig. 2. Differentially expressed genes in ARID1A KDs are dysregulated in OCCC. Expression changes were assayed from two biological replicates of each of the two KD lines and the SCR line using Illumina BeadArray. (a) The expression of 99 genes were significantly (Log_2 (Fold Change) > 0.5; p-value < 0.05) affected by ARID1A downregulation. (b) Oncomine™ heatmap analysis of the publicly available Hendrix dataset [53] comparing normal ovary (Normal) to OCCC (Tumor) for expression of genes altered by ARID1A downregulation. Genes are ordered by p-value of the differential expression between normal/tumor. Twenty genes either down-regulated (top) or up-regulated (bottom) in KD cell line are shown. (c) Canonical pathways affected by differentially expressed genes as identified by Ingenuity Pathway Analysis (IPA). The length of the bar connotes significance. (p-value < 0.05). Pathways involved in cancer are highlighted in blue and those involved in immune response are highlighted in pink. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Thus, these data show that genes exhibiting expression changes due to down-regulation of ARID1A in immortalized endometriosis cells function in pathways and networks critical to tumorigenesis and are frequently altered in primary ovarian tumors.

Global chromatin accessibility and DNA methylation signatures are maintained after decreased ARID1A

Due to its potential role as a chromatin remodeler protein, the decreased ARID1A levels may affect chromatin architecture and contribute to the observed phenotypic and gene expression alterations. We used the NOMe-seq assay to simultaneously evaluate whole genome assessment of chromatin accessibility and DNA methylation [21,22]. This method uses a GpC methyltransferase to methylate GpC sites present in DNA regions that are free of nucleosomes or other DNA binding proteins [39,40]. Upon bisulfite conversion and sequencing, NOMe-seq delivers a precise digital readout of both endogenous DNA methylation at the CpG sites and nucleosome occupancy or chromatin accessibility from the GpC sites.

Firstly, we interrogated chromatin accessibility and DNA methylation levels at aligned transcription start sites (TSSs) and CTCF insulator binding sites to attain a global overview of the effect of ARID1A down-regulation. Consistent with the literature, the majority of CpG Island (CGI) promoters were unmethylated and maintained a strong nucleosome depleted region (NDR) upstream of the TSS in both SCR and KD cell lines [21] (Fig. 3, top panel). In contrast, most of the non-CGI promoters were methylated and NDRs were present in only a small subset of unmethylated non-CGI promoters (Fig. 3, middle panel). Finally, the well-positioned and highly regular anti-correlative phasing of nucleosomes and DNA methylation surrounding CTCF binding sites [21,22] was also maintained in the ARID1A KD cell line (Fig. 3, bottom panel). Thus, in spite of the reduction of ARID1A expression in iEEC16 cells and the resulting phenotypic and expression changes, the global chromatin accessibility or DNA methylation signatures at TSSs or CTCF insulator sites is retained (Fig. 3).

ARID1A loss contributes to reconfiguration of chromatin through the widespread redistribution of H3K27ac and modests alteration of H3K27me3 at promoters

Since we did not detect global deregulation of accessibility or methylation at gene promoters and CTCF sites, we coupled our

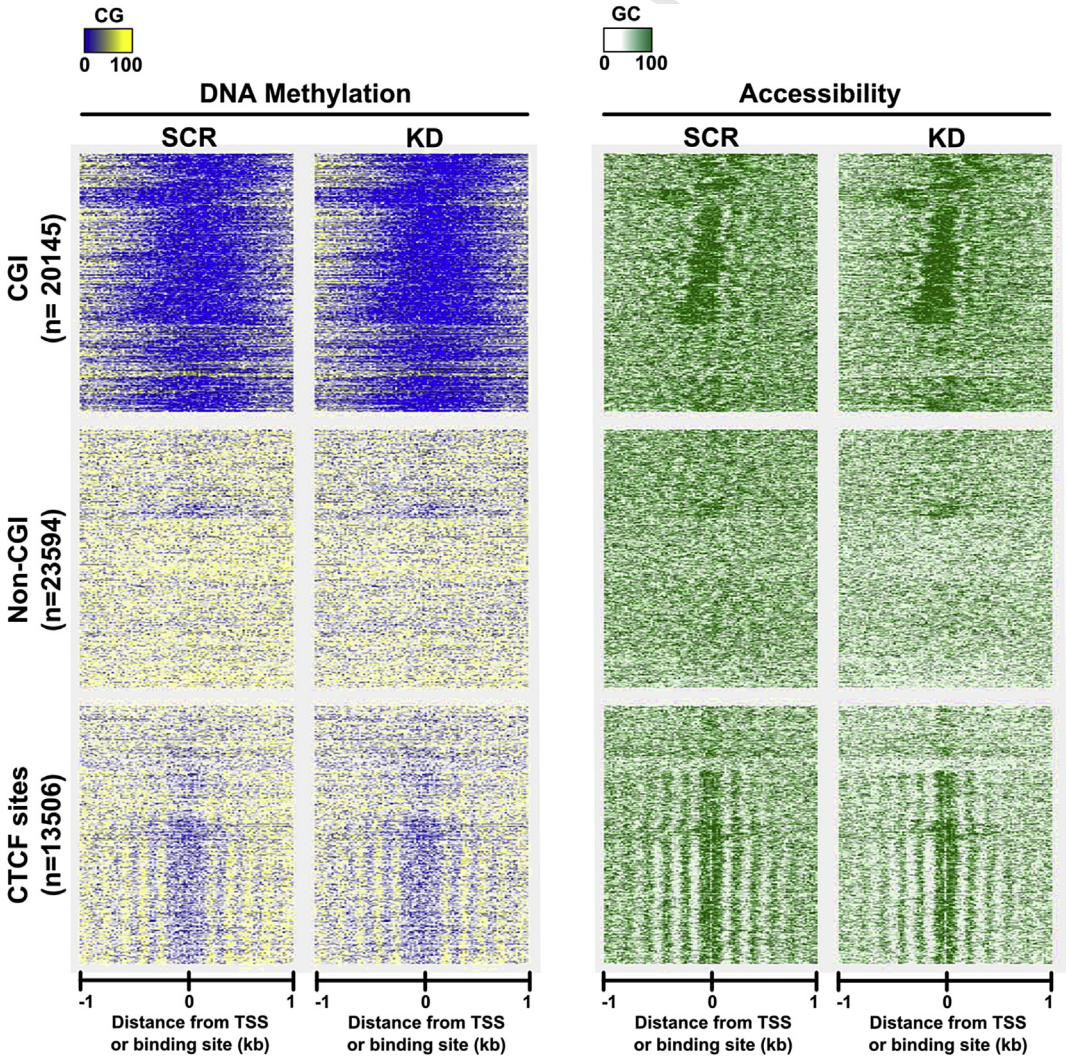

Fig. 3. NOMe-seq reveals that the majority of the genome is unperturbed by down-regulation of ARID1A. Heatmaps of NOMe-seq methylation levels (CG methylation for "DNA methylation" and GC methylation for "Accessibility") were aligned to the center of CGI TSS (N = 20,145), non-CGI TSS (N = 23,594) or CTCF binding sites (N = 13,506), extended by ± 1 kb. CpG methylation is plotted on a scale of 0 (blue) to 100 (yellow) and GpC methylation is plotted on a scale of 0 (white) to 100 (green). TSS or CTCF sites were hierarchically clustered based on the accessibility within ± 250 bp of the site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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NOMe-seq data with ChIP-seq data of various histone modifications to segment the genome into functional regions. Previous reports have indicated that H3K27ac and H3K27me3 marks might be involved in mediating ARID1A activity [41–44], therefore, we focused on the variations observed in these modifications.

While global levels of H3K27ac and H3K27me3 were only marginally altered in response to ARID1A KD in iEEC16 cells (Supplemental Fig. 2), when the H3K27ac and H3K27me3 peaks were segmented into regulatory elements such as promoter and enhancers, a striking pattern emerged. The majority (>60%) of the H3K27ac promoter peaks showed greater read-count enrichment in the KD cells, while less than 0.5% of the promoter peaks were depleted in the KD cells (p-value < 2E-5) (Fig. 4a). To understand the functional relevance of this altered H3K27ac distribution on the rest of the epigenetic landscape of iEEC16 cells, we examined the top 10% most altered H3K27ac promoter peaks, corresponding to 711 TSSs, by overlapping them with the NOMe-seq data. While the promoters in both SCR and KD cells were unmethylated and most possessed an NDR upstream of the TSSs (Supplemental Fig. 3a), a subset of the promoters showed a more pronounced region of accessibility in KD cells (Supplemental Fig. 3a, marked by orange box) suggesting that ARID1A mediates targeted, rather than global, chromatin remodeling.

In contrast to H3K27ac at promoters, we found that only 15% of the H3K27me3 promoter peaks were altered in read count enrichment in ARID1A KDs; 8.5% showed greater enrichment and 6.5% showed depletion of this repressive mark in the KD cell line (Fig. 4b). As with H3K27ac peaks, overlaying NOMe-seq data on the H3K27me3 peaks showed no changes in DNA methylation (Supplemental Fig. 3b; left two panels). However, a small subset of gene promoters losing H3K27me3 (Supplemental Fig. 3b; right two panels, orange box) showed a modest gain in chromatin accessibility, while the promoters gaining H3K27me3 in the KD cell line displayed a decrease in accessibility in these regions (Supplemental Fig. 3b; right two panels, below the dotted line). Thus, the down-regulation of ARID1A in endometriosis cells reconfigures chromatin architecture through extensive increase of H3K27ac, and a modest, context-dependent alteration of H3K27me3 at gene promoters.

Down-regulation of ARID1A also contributes to the redistribution of H3K27ac mark at enhancers

In contrast to the increased enrichment of H3K27ac at most promoters, the majority of the altered enhancer peaks were reduced in H3K27ac upon the down-regulation of ARID1A (Fig. 4c). Nearly 24% of the enhancer peaks were decreased and less than 5% of the enhancer peaks were increased in H3K27ac enrichment in the KD cells (p-value < 2E-5) (Fig. 4c).

Unlike promoters, enhancers do not have a defined start site with which to align NOMe-seq data. Therefore, to generate chromatin accessibility and methylation maps of the enhancers (Supplemental Fig. 3c) we aligned the top 10% of the most altered H3K27ac enhancer peaks with NDRs identified from NOMe-seq data using a Hidden Markov Model (HMM) approach previously described [21,22]. Nearly all of the enhancer peaks contained NDRs in SCR sample. Although no notable changes in DNA methylation were observed across the samples (Supplemental Fig. 3c, left 2 panels), a subset of these enhancers lost NDRs in the KD (Supplemental Fig. 3c, left 2 panels, marked by orange box), consistent with the loss of an active mark and suggesting that these regions undergo DNA methylation-independent chromatin remodeling upon ARID1A downregulation. These findings, coupled with those at promoters, suggest that ARID1A plays an important role in the distribution of H3K27ac histone marks and is driving changes to nucleosome positioning in these regions in a targeted and DNA methylation-independent manner.

The majority of differentially expressed genes are directly affected by epigenetic changes due to the decrease of ARID1A

Next, we sought to elucidate the ARID1A-mediated epigenetic regulation of the genes differentially expressed in ARID1A knockdown cells. We examined the chromatin accessibility data at all dysregulated gene promoters and putative enhancers (see Methods). By averaging the accessibility across the promoters, we found a small but significant correlative loss of chromatin accessibility consistent with gene expression at the 77 downregulated genes (Supplemental Fig. 4a and 4c) and a correlative gain in accessibility at the 27 upregulated genes (Supplemental Fig. 4b and 4c). Likewise, a trend of accessibility loss consistent with gene downregulation was noted at the 51 putative enhancers (Supplemental Fig. 4d and 4f), and a trend of accessibility gain consistent with gene upregulation was noted at the 18 putative enhancers (Supplemental Fig. 4e and 4f) though these changes were not statistically significant. Additionally, we observed that approximately 60% of genes changing in expression also had an accompanying change in histone marks at regulatory elements upon ARID1A KD (Fig. 5, Supplemental Table 3). Among the down-regulated genes, nine promoters displayed loss of H3K27ac, nine promoters displayed gain of H3K27me3, and 39 genes showed loss of H3K27ac at a putative enhancers (Fig. 5a), which correlate with expected chromatin-mediated gene downregulation. Conversely, among upregulated genes, 13 promoters saw gain of H3K27ac, nine promoters saw a loss of H3K27me3, and seven genes gained acetylation at a putative enhancers (Fig. 5b), which likewise correlate with chromatin-mediated gene upregulation. Thus, these complementary active and repressive mark changes could be mediating the expression changes of the respective genes. The remaining genes with minimal to no histone marker changes could be due to secondary histone modification-independent effects, such as variations in the abundance of transcriptional factors in ARID1A KD cells.

As an illustration of ARID1A-driven epigenetic changes at promoters affecting gene expression, the *SVIL* and *ZNF583* promoters showed a gain of the active H3K27ac mark and both of these genes were increased in expression in the KD lines. In addition, the gain of H3K27ac mark at *SVIL* promoter was complemented with decreased H3K27me3 (Fig. 6a). Overlaying ChIP-seq data with locus-specific NOMe-seq data at the *ZNF583* CpG island promoter (Fig. 6c), we observed an overall increase in accessibility directly upstream of the TSSs along with a gain of H3K27ac peak in the KD cells (Fig. 6c, highlighted with blue boxes). Furthermore, the nucleosomes were less consistently maintained in the KD cell line indicating weaker positioning relative to the SCR cell line. Thus, ARID1A may be required for the down-regulation of *ZNF583* expression by maintaining strongly positioned nucleosomes at its promoter. In accordance meanwhile, the promoters of down-regulated genes *SULF1* and *CCL2* showed H3K27ac depletion in the KD cells, and the *SULF1* promoter was also enriched for the repressive H3K27me3 mark (Fig. 6b).

Similarly modulation of active marks at putative enhancers also seem to mediate gene expression changes. For example, enhancer peaks corresponding to the upregulated genes *LRP3* and *JAM3* displayed an increase in the enrichment of H3K27ac in the KD cells (Fig. 6d), while the corresponding enhancers to the downregulated genes *TLE* and *NTF3* were strongly depleted in the KD (Fig. 6e). *NTF3* notably was downregulated in both the iEEC16 cell line (Supplemental Table 2) and in primary OCCC expression data from Oncomine™ (Fig. 2b, top panel). Locus-specific NOMe-seq analysis

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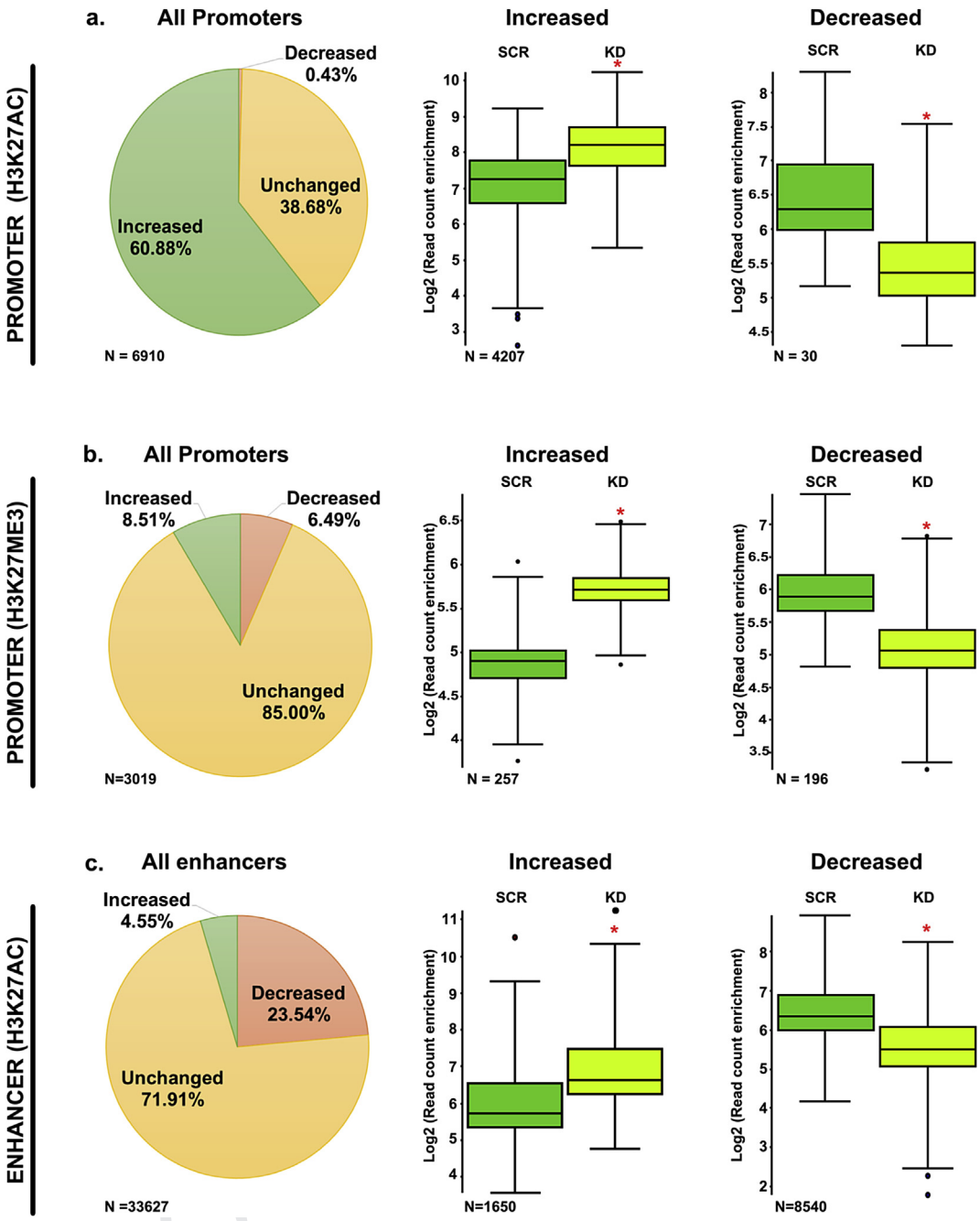

Fig. 4. Down-regulation of ARD1A promotes re-distribution of H3K27ac and H4K27me3. (a–c) H3K27ac and H3K27me3 data from ARID1A KD and SCR cell lines were used. Pie-charts summarize histone peaks with increased read-count enrichment by 1.5-fold ("increased"), decreased read-count enrichment by 1.5-fold ("decreased"), or unchanged in read-count enrichment ("unchanged") in the KD cells relative to SCR cells. Box-plots quantify log2 of read-count enrichment in the "increased" or "decreased" peaks. (a) H3K27ac peaks at gene promoters (n = 6910) (b) H3K27me3 peaks at gene promoters (n = 3019). (c) H3K27ac enhancer peaks (n = 33,627). (p-value < 2E-5).

of the *NTF3* enhancer revealed a stark loss in accessibility in the KD cells (marked by a green line in Fig. 6f), which could result in the decreased *NTF3* gene expression observed in the KD cell lines. Thus, ARID1A could be required to keep the chromatin open and accessible in the enhancer for proper transcription and expression of *NTF3*.

In summary, nearly 60% of the genes differentially expressed by ARID1A KDs were also correspondingly altered in active and repressive histone marks in promoters and/or enhancers (Fig. 5a and 5b). Closer inspection of some of these regulatory elements showed stark changes in chromatin accessibility complementing the histone modification and gene expression variations (Fig. 6c and 6f). Taken together, these data suggest that gene expression changes resulting from loss of ARID1A is partly mediated by DNA methylation-independent modulation of histone modifications and chromatin accessibility.

Discussion

OCCC is an aggressive subtype of EOC that is tightly correlated with preexisting endometriosis. *ARID1A* gene mutations and loss of expression are common to both OCCC and endometriosis.

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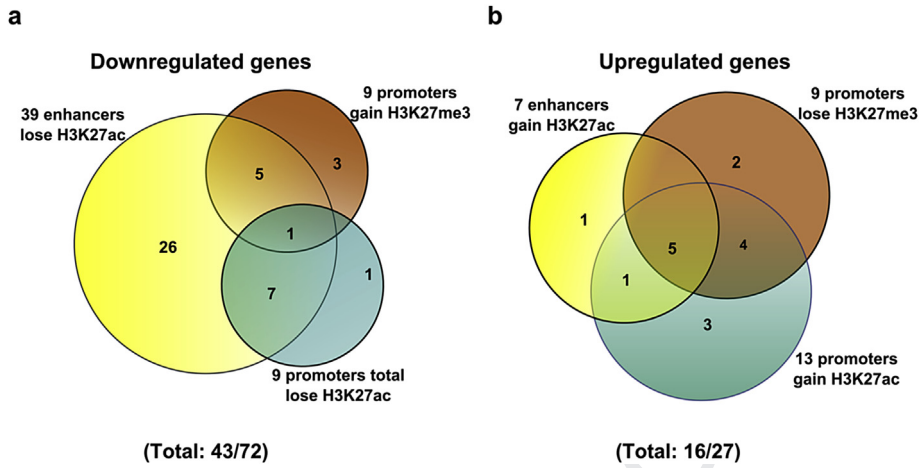

Fig. 5. A subset of the differentially expressed genes are epigenetically regulated by ARID1A. (a) Venn-diagrams of down-regulated genes showing any loss in H3K27ac (at promoters or enhancers) or gain in H3K27me3 at gene promoters. (b) Venn-diagrams of up-regulated genes showing any gain in H3K27ac (at promoters or enhancers) or loss of H3K27me3 at gene promoters.

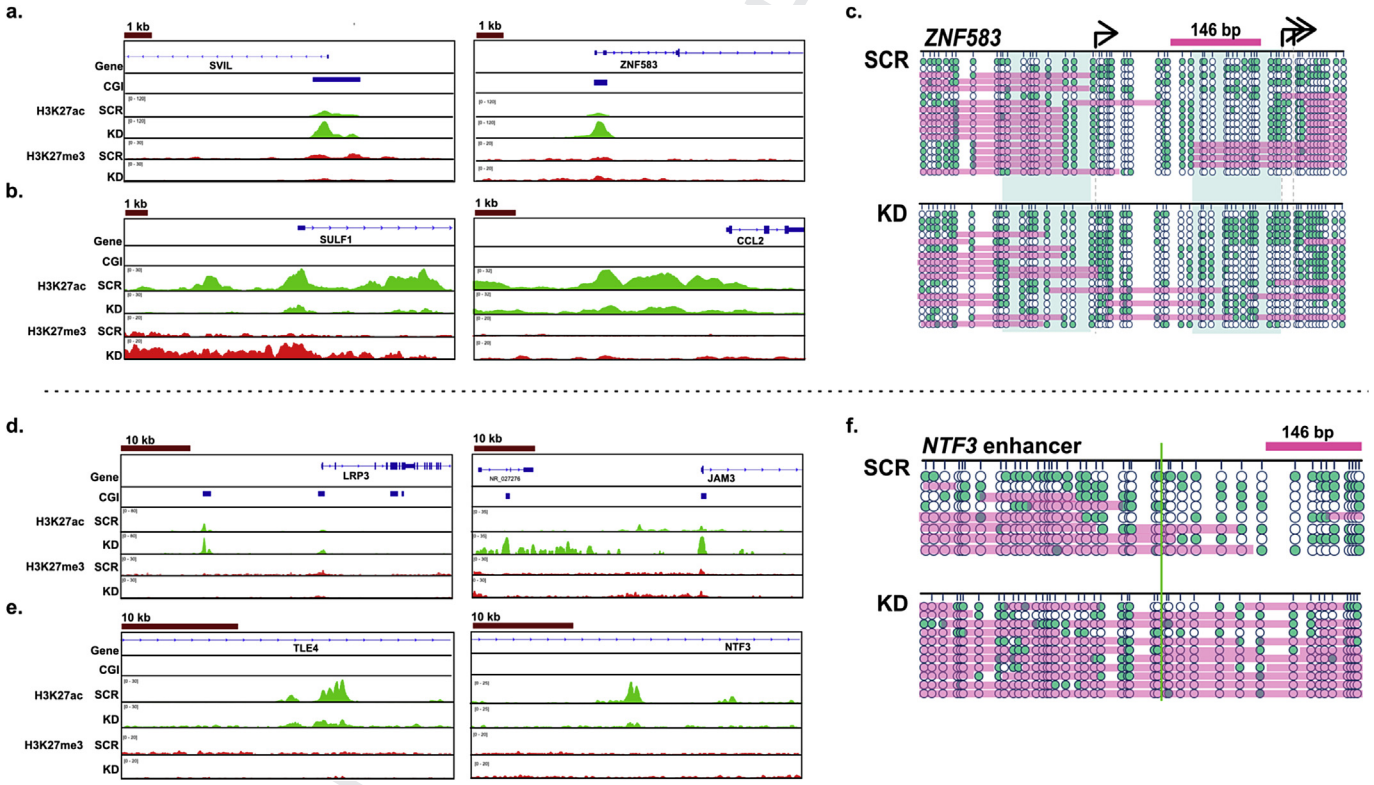

Fig. 6. Down-regulation of ARID1A contributes to both gain and loss of regulatory elements. IGV browser view of gene promoters and enhancers (a, b, d, e). The first track shows the gene or genomic region, followed by a track denoting CGIs. Next, wiggle plots of H3K27ac ChIP-seq enrichment in SCR cells and ARID1A KD cells are shown, finally wiggle plots of H3K27me3 ChIP-seq enrichment data are displayed. (c, f) Unfilled circles are unmethylated GpC sites, teal filled circles denote methylated (accessible) GpC sites. Nucleosome is indicated with a pink bar representing region of inaccessibility greater than 146bp. TSS is marked by black arrow and center of enhancer peak is denoted by a green line. (a) *SVIL* and *ZNF583*, are gaining acetylation peak at their respective promoters, and (b) *SULF1* and *CCL2*, are losing the H3K27ac mark at the promoter. (c) The gain in H3K27ac at the *ZNF583* promoter is accompanied by increased chromatin accessibility upstream of the 3 TSSs. (d) *LRP3* and *JAM3*, gain acetylation peak at their respective gene body enhancer, and (e) *TLE4* and *NTF3* lose the active enhancer element in the gene body. (f) The decrease in H3K27ac at the enhancer of *NTF3* is accompanied by decreased chromatin accessibility downstream of the enhancer peak center. ADDIN. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Therefore, in this study, we characterized the effects of ARID1A down-regulation in endometriosis cells to identify the phenotypic and molecular changes that can potentially contribute to the malignant transformation of endometriotic cells to OCCC. First, we observed higher efficiency of anchorage-independent growth, increased cell propensity to adhere to collagen, and increased invasion of basement membrane *in vitro*. Anchorage-independent growth is known to correlate with neoplastic growth and considered to be a barometer of tumorigenicity [45,46]. Additionally, cell adhesion to collagen can be crucial for cancer cell signaling, invasion, and metastasis [47,48]. Thus, the phenotypic alterations due to ARID1A loss in the non-tumorigenic iEEC16 cell line suggest that

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ARID1A mutation could be a necessary step in the malignant transformation of endometriotic cells.

Gene expression analysis revealed that nearly 100 genes were altered in expression upon down-regulation of ARID1A. A majority of these expression changes were accompanied by alterations in histone modification. However, gene transcription is controlled by the availability of regulatory factors and transcriptional machinery, along with the permissiveness of regulatory elements (marked by active histone marks). A recent study suggested that loss of ARID1A can restrict promoter accessibility to transcription factors, thereby affecting histone modifications, and in turn effecting gene expression and phenotypic changes [43]. It has also been noted that SWI/SNF complexes can compensate for the loss of ARID1A by incorporating homologs such as ARID1B [43,49]. These secondary mechanisms may also contribute to expression changes, especially of the genes for which minimal to no histone modification alterations are evident.

One of the key findings from this work is that ARID1A mediated effects on chromatin structure are context- and locus-dependent, rather than global. This localized effect may be due to the various binding partners at these regulatory elements. These data are supported by a recent study investigating the consequences of ARID1A loss in colon cancer [44], which found that ARID1A is necessary for the proper steering of SWI/SNF complexes to its specific genomic targets. Particularly, the group noted minimal alteration of H3K27ac enrichment at promoters and a significant diminishing of H3K27ac levels at enhancers in the absence of ARID1A correlating with reduced mRNA levels of the nearest genes. ARID1A is known to function at both promoters and enhancers, and SWI/SNF complex members have been found to interact with both histone acetyltransferases (HATs) and histone deacetylases (HDACs) [50–52]. Accordingly, we noted that over 60% of promoters and nearly 25% of enhancers were altered in H3K27ac enrichment upon loss of ARID1A, and some of these histone changes translated to stark corresponding alterations in chromatin accessibility, and likely gene expression, as the majority of the differentially expressed genes showed correlative histone mark alterations. Furthermore, we noted substantial chromatin accessibility modulation in a locus specific manner. Thus, we find ARID1A has a targeted, rather than global, effect on the epigenome and transcriptional regulation. Together, these findings highlight the context-dependent role of chromatin remodelers in normal and cancerous tissue.

In summary, our findings demonstrate that reduced ARID1A alters the distribution of histone modifications, modulates the permissiveness of some regulatory elements, affects gene expression changes, and finally contributes to cellular transformation. It has been long speculated that endometriosis could be a precursor to OCCC [7,15], and our work provides evidence that loss of ARID1A in non-tumorigenic endometriosis cells is sufficient for the induction of epigenetic, molecular, and phenotypic alterations indicative of potentially oncogenic transformation.

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Conflict of interest

P.A. Jones is a paid consultant of Zymo Research Corporation, but otherwise the authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.canlet.2017.04.040.

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