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ARID3a gene profiles are strongly associated with human interferon alpha production

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ABSTRACT

Type I interferons (IFN) causes inflammatory responses to pathogens, and can be elevated in autoimmune diseases such as systemic lupus erythematosus (SLE). We previously reported unexpected associations of increased numbers of B lymphocytes expressing the DNA-binding protein ARID3a with both IFN alpha (IFNα) expression and increased disease activity in SLE. Here, we determined that IFNα producing low density neutrophils (LDNs) and plasmacytoid dendritic cells (pDCs) from SLE patients exhibit strong associations between ARID3a protein expression and IFNα production. Moreover, SLE disease activity indices correlate most strongly with percentages of ARID3a$^+$ LDNs, but were also associated, less significantly, with IFNα expression in LDNs and pDCs. Hierarchical clustering and transcriptome analyses of LDNs and pDCs revealed SLE patients with low ARID3a expression cluster with healthy controls and identified gene profiles associated with increased proportions of ARID3a- and IFNα-expressing cells of each type. These data identify ARID3a as a potential transcription regulator of IFNα-related inflammatory responses and other pathways important for SLE disease activity.

1. Introduction

ARID3a (A-T rich interacting domain 3a) is a DNA-binding protein that modulates gene expression, increasing immunoglobulin gene expression in B lymphocytes and repressing expression of other genes in neonatal fibroblasts [1–3]. We previously described increases in the number of circulating ARID3a$^+$ B lymphocytes in SLE patients, and found that these cells are associated with increases in disease activity indices as defined by SLEDAI scores (SLE disease activity indices) [4]. Others found that B lymphocytes produce Type I IFNs during development and in response to infections [5–7]. We have also shown that induction of ARID3a protein in healthy B lymphocytes results in Type I IFN production [8], suggesting an unexpected link between ARID3a and IFN. Furthermore, SLE patient B lymphocytes with high levels of ARID3a expression also exhibited increases in IFN signature gene expression compared to healthy controls and SLE B cells with low ARID3a expression levels [8,9]. Together, these data suggest that ARID3a expression in both SLE and healthy B cells is associated with IFN production.

In healthy individuals, Type I IFNs are essential for immune responses against intracellular pathogens, including viruses that trigger anti-DNA and anti-RNA responses [10]. Virtually all cells can produce and respond to IFNs, with differing cell type-specific responses that can be detrimental [11]. Increased plasma Type I IFN, mainly IFNα, occurs in approximately half of adult SLE patients, and is associated with increased disease activity [12–16]. IFNα expression in autoimmune diseases results in different gene signatures than those produced by viral infections [17], and dysregulated IFNα levels in SLE activate a panel of

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IFN-regulated genes, commonly referred to as an IFN signature [18]. Transcriptomic analyses of whole blood from pediatric SLE patients further defined this gene signature [19]. Although increases in IFNα accelerated disease onset [20–22], and depletion of major subsets of IFNα-producing cells ameliorated autoimmunity in several mouse models [23,24], the reasons for elevated IFNα expression in SLE patients is unknown. Several clinical trials using treatments that inhibit IFN production as therapies for SLE are ongoing, [reviewed in ref. [25]]. Thus, understanding underlying mechanisms associated with increased IFNα production is important.

Plasmacytoid dendritic cells (pDCs) are thought to be a major source of IFNα in SLE patients, [reviewed in ref. [26]]. Other cell types, including neutrophils [27–29], also contribute to IFNα production in SLE. A population of low density neutrophils (LDNs) present in the peripheral blood mononuclear fraction of pediatric SLE patient samples have enhanced capacity to synthesize IFNα [29], contributed to tissue damage in adult SLE patients [30,31], and were also associated with increased disease activity in SLE [30,32,33]. We demonstrated that healthy, ARID3a$^+$ B lymphocytes induced both IFN production and ARID3a expression in autologous pDCs [8]. These data led us to hypothesize that ARID3a might be a biomarker for IFN production in pDCs and other cell types, including LDNs.

Nothing is known regarding ARID3a expression in pDCs and LDNs, including whether its expression is correlated in these cells with increased IFN production. Therefore, we assessed levels of intracellular ARID3a and IFNα in pDCs and LDNs from healthy controls and SLE patients with a wide range of disease activity. ARID3a protein levels correlated with IFNα levels in both pDCs and LDNs. To identify genes associated with ARID3a expression in LDNs and pDCs, RNA-seq was performed and expressed genes were correlated with ARID3a protein levels. Our data define gene profiles associated with ARID3a and IFN expression in both pDCs and LDNs and implicate ARID3a as a regulator of innate immune responses in these cells.

2. Materials and methods

2.1. Patients and healthy controls

Healthy age and sex-matched controls and patients who met a minimum of four American College of Rheumatology Classification Criteria for SLE [34] were recruited after informed consent from the Oklahoma Medical Research Foundation (OMRF) Oklahoma Lupus Center in accordance with OMRF (IRB compliance #06-19) and OUHSC (IRB compliance 5946) Institutional Review Board approvals and in accordance with the Declaration of Helsinki. SLE patients (27) ranging in age from 21 to 73 (96% female, SLEDAI 0 to 8, Table 1) and 11 healthy controls were recruited for this study. All patients were under treatment regimens at blood draw (Table 1).

2.2. Sample preparation

Total peripheral blood mononuclear cells (PBMCs) from patients and healthy controls were isolated from heparinized peripheral blood with Ficoll Paque Plus (GE Healthcare, Fisher Scientific cat# 45-001-749). Two million PBMCs were stained for flow cytometric analysis and the remaining cells were either cryopreserved for later use or were immediately enriched as described below for either LDNs or pDCs. Patient plasma samples were stored at −80 °C until assay. LDNs were isolated for RNA analyses from either cryopreserved or freshly isolated PBMCs using the EasySep Human Neutrophil Enrichment Kit (cat# 19257, Stemcell Technologies) according to manufacturer instructions. Isolation of pDCs was performed using the human Plasmacytoid Dendritic Cell Isolation Kit II (cat# 130-097-415, Miltenyi Biotec) according to manufacturer instructions using LD columns (cat# 130-042-901, Miltenyi Biotec). Patient samples chosen for RNA analyses were based on population cell numbers and ARID3a expression levels identified by flow cytometric evaluation of total PBMCs.

Table 1
Study subject demographics.

Subject	Race	Gender	Age	SLEDAI	Treatment
SLE 1	C	F	71	4	HCQ
SLE 2	C	F	73	3	HCQ
SLE 3	C	F	62	0	HCQ, MTX
SLE4	C	F	71	0	AZA
SLE 5	AA	F	51	6	HCQ, RTX
SLE 6	C	F	43	8	PDN,Q
SLE 7	AA	F	34	5	HCQ
SLE 8	NA/H	F	29	4	AZA, HCQ
SLE 9	AA	F	32	4	HCQ
SLE 10	AA	M	56	6	PDN, AZA, HCQ
SLE 11	H	F	39	0	MTX, HCQ, PDN
SLE 12	A	F	52	0	AZA
SLE 13	C	F	36	1	AZA, HCQ
SLE 14	C	F	34	2	MTX, HCQ, PDN
SLE 15	AA	F	18	0	PDN, AZA, HCQ
SLE 16	A	M	35	6	HCQ
SLE 17	AA	F	21	2	MMF, PDN, HCQ
SLE 18	C	F	28	2	PDN, HCQ
SLE 19	C	F	45	6	HCQ, RTX
SLE 20	AA	F	42	2	PDN, HCQ
SLE 21	C	F	64	2	PDN, HCQ, HC
SLE 22	AA	F	59	6	MTX, HCQ
SLE 23	AA	F	50	4	MMF, ADA, HCQ
SLE 24	AA	F	36	4	PDN, HCQ
SLE 25	AA	F	53	4	HCQ, RTX, PDN
SLE 26	C	F	55	2	MTX, HCQ, B
SLE 27	C	F	62	0	PDN, AZA, HCQ
C1	C	F	48	N/A	
C2	C	F	55	N/A	
C3	C	F	35	N/A	
C4	C	F	46	N/A	
C5	C	M	57	N/A	
C6	C	F	50	N/A	
C7	C	F	34	N/A	
C8	AA	F	63	N/A	
C9	AA	F	40	N/A	
C10	C	F	44	N/A	
C11	C	F	33	N/A	

SLE patients (SLE) and healthy controls (C) are listed. Races are: A- Asian, AA-African American, C- Caucasian, H-Hispanic, NA - Native American. Age at time of blood draw and gender (F-female, M-male) are indicated. Disease activity indices (SLEDAI scores) and drug treatments are indicated for patients. Drugs: HCQ-hydroxychloroquine, MTX-methotrexate, AZA- Azathioprine, RTX-Rituximab, PDN-Prednisone, HC- Hydrocortisone, ADA- Humira, B- Benlysta, MMF-Cellcept, Q-Quinacrine.

2.3. Flow cytometry analyses

pDCs were defined as CD3$^-$CD20$^-$CD56$^-$CD11c$^-$CD123$^+$CD304$^+$ [35,36]. LDNs were defined as CD3$^-$CD20$^-$CD56$^-$CD14$^-$CD15$^+$CD16$^+$CD16b$^+$ [32]. The use of CD16b was used as a marker to ensure exclusion of NK cells [37] that may express ARID3a (our unpublished data). Appropriate matching isotype controls from Biolegend were used for gating. Following surface marker staining, cells were fixed with fixation buffer (BD Biosciences), permeabilized with Transcription Factor Fixation/Permeabilization Buffer kit (eBioscience) and stained for IFNα and ARID3a. IFNα-Phycoerythrin (Clone LT27:295, cat# 130-092-601) recognizes the majority of the IFN-α subtypes, but not IFN-α2b. Human-specific anti-ARID3a antibodies were generated in goats by our laboratory against a peptide sequence from the amino terminal portion of ARID3a (G-R-G-R-E-G-P-G-E-E-H-F-E), and were purified over a peptide column, verified by western blot and mobility shift against *in vitro* translated human ARID3a and B cell nuclear extracts containing ARID3a [1,38]. A rabbit anti-goat Fluorescein (Cat# 6160-02) was used as the secondary antibody. Doublet exclusion was used to ensure analyses of single cells prior to forward/side scatter gating. Data

were collected using an LSRII (BD Biogenics) and FACSDiva (BD Biosciences) software version 4.1 or Stratedigm S1200Ex and CellCapTure acquisition software and were analyzed using FlowJo (Tree Star) software version 10. Specific antibodies used were: human lineage markers CD3 Pacific Blue (clone UCHT1, cat# 300434), CD20 Pacific Blue (Clone 2H7, cat# 302330), and CD56 Pacific Blue (Clone 5.1H11, cat# 362552), CD11c-Brillian Violet 605 (Clone 3.9, 301636), CD16-Allophycocyanin-Cyanin7 (Clone 3G6, cat#302018), CD14-Allophycocyanin (Clone 63D3, cat#367118), CD304-Brilliant Violet 510 (Clone 12C2, cat# 354515), CD15-Brilliant Violet 605 (Clone W6D3, cat# 323032), and CD19-Phycoerythrin-Cyanin5.5 (Clone HIB19, cat# 302210) from Biolegend, CD123-Allophycocyanin-Vio770 (Clone AC145, cat#130-104-196) from Miltenyi Biotec, and CD16b-Alexa fluor700 (Clone 245514, cat# FAB1597N) from R&D Systems.

2.4. RNA-seq

RNA from enriched LDNs and pDCs was isolated using the MagMAX mirVana Total RNA Isolation Kit (Applied Biosystems, cat# A27828) according to manufacturer instructions. RNA concentrations were measured with an Impen Nanophotometer. RNA samples were prepared for sequencing using the Ovation RNA-Seq v2 (NuGEN Technologies) kit and libraries were constructed using Ovation Ultralow Library System V2. Library concentration and fragment size distribution was determined using Agilent High Sensitivity D1000 kit on an Agilent 2200 TapeStation (Agilent Technologies). Paired-end (2 × 75bp) sequencing was performed on a NextSeq platform using SBS v2 chemistry.

Fastq files were analyzed using FastQC and low quality reads and sequencing adapters were trimmed using Trimmomatic [39]. A Bowtie index of raw FASTQ files was created based on the UCSC knownGene (hg38) transcriptome, and paired-end reads were aligned directly to this index using default parameters [40]. RSEM [41] was run using default parameters on the aligned reads to estimate gene expression levels. Library quality metrics, such as genomic mapping rates, library and the fraction of ribosomal RNA in each library were calculated. Two SLE pDC, one healthy control pDC, and one SLE neutrophil samples were excluded due to low library size and poor alignment. Differential gene expression was analyzed using limma [42]. Unsupervised hierarchical clustering was performed on differentially expressed genes (FDR < 0.05). Pathway analyses were performed using Ingenuity Pathway Analysis (Qiagen). Heatmaps in Fig. 4 were constructed of all genes that significantly correlated with % IFNα$^+$ or % ARID3a$^+$ cells by Spearman's correlation (unadjusted $p < 0.05$). All correlation coefficients of all top genes with respect to each other and % protein-expressing cells were then plotted using the unsupervised clustering heatmap R package corrplot.

2.5. Plasma IFNα activity

The WISH endothelial cell line (ATCC, CCL-2; gift from S. Kovats) that expresses IFNα receptors, but cannot use endogenous IFN pathways, was used to measure IFN-responsive gene activation [43,44]. WISH cells (50,000 cells/well) were cultured 1:2 with SLE patient or control plasma, or recombinant human IFNα2a (Invitrogen, cat# 111001) at 75U per well, in DMEM supplemented with 10% FBS for 6 h at 37 °C prior to lysis for RNA isolation with Tri-reagent (Sigma, cat# T9424). RNA concentration was determined by Implen Nanophotometer. cDNA synthesis was performed using iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad, cat# 1725034). qPCR was performed on a Bio-Rad CFX96 real time PCR machine with the following program: 1: 1 min at 95 °C, 2: 10 s at 95 °C, 3: 30 s at 57 °C, repeat 2 & 3 40 times, hold at 4 °C. Primers for *IFI44* and *HPRT* were purchased from IDT [43]. *IFI44* forward primer: 5′CTC GGT GGT TAG CAA TTA TTC CTC 3′, reverse primer: 5′AGC CCA TAG CAT TCG TCT CAG 3'. *HPRT* forward primer: 5′ TTG GTC AGG CAG TAT AAT CC 3′, reverse primer: 5′GGG CAT ATC CTS CAA CAA AC 3'. Data were normalized to *HPRT1*. Fold-increases were calculated relative to the values of *IFI44* for unstimulated cells.

2.6. Data analyses

Simple linear regression analyses of SLEDAI scores and proportions of cells producing ARID3a and IFNα were performed by best fit linear regression using Prism (Graphpad) version 7 ($p < 0.05$ indicates the slope of the best-fit line differs from 0). Data was log-transformed where indicated to satisfy assumptions of normality. For RNA-seq data, genes with FDR-adjusted p values of less than 0.05 from Limma were considered differentially expressed. Genes that were expressed at a transcript per million (TPM) of greater than one in at least one sample were kept for downstream analysis. TPM values were log transformed. For correlations with protein expression, TPM values were correlated with % ARID3a$^+$ or % IFNα$^+$ cells using Spearman's Correlation (R statistical software, p values were not adjusted). A subset of those genes was also analyzed by linear regression. Stepwise additive and subtractive multiple linear regression models of SLEDAI were performed independently for pDCs and LDNs using the R function stepAIC within the MASS package.

2.7. Data sharing statement

RNA-seq data are publicly available through the GEO NCBI database under the accession number GSE117836. For original data, contact carol-webb@ouhsc.edu.

3. Results

3.1. Association of ARID3a and IFNα protein expression in LDNs and pDCs

Healthy controls and SLE patients with a range of SLEDAI scores from 0 to 8 were recruited (Table 1). Total PBMCs were subjected to flow cytometry using surface markers to identify LDNs and pDCs, and intracellular staining identified IFNα and ARID3a protein expression (Fig. 1A, B). T lymphocytes typically do not express ARID3a or IFNα, and served as internal controls for intracellular staining integrity for each PBMC sample (Fig. 1C). Healthy controls showed fewer than 20% ARID3a and IFNα-expressing LDNs (Fig. 1D). However, ARID3a protein expression correlated strongly with IFNα expression ($R^2 = 0.64$, $p < 0.0001$) in LDNs of SLE patients (Fig. 1D). Similarly, in pDCs from SLE patients, ARID3a and IFNα protein expression were strongly correlated ($R^2 = 0.81$, $p < 0.0001$), while healthy controls had few cells expressing either protein (Fig. 1E). Interestingly, increased percentages of ARID3a-expressing LDNs were found in SLE patients with increased disease activity indices (SLEDAI scores) by univariate linear regression (Fig. 1F), while disease activity was less strongly associated with percentages of ARID3a$^+$ pDCs (Fig. 1G). Surprisingly, while ARID3a and IFNα protein levels are correlated in both pDCs and LDNs, ARID3a expression in LDNs is more significantly associated with disease activity scores.

3.2. IFNα expression is weakly associated with disease activity

We next assessed associations of IFNα protein expression levels in LDNs and pDCs with increased disease activity scores. Only poor correlations with SLEDAI scores were observed for IFNα expression in the LDNs (Fig. 2A), unlike what we observed for ARID3a (Fig. 1F). Furthermore, IFNα expression was only weakly associated with SLEDAI scores in pDCs (Fig. 2B). Evaluation of IFN signatures and IFNα plasma activity (represented by *IFI44* gene expression) revealed weak associations with numbers of IFNα-expressing LDNs or pDCs (Fig. 2C and D). Likewise, IFNα plasma activity was only weakly associated with numbers of ARID3a-expressing LDNs or pDCs (Fig. 2E and F). Similar results were found using the *IFIT1* gene (not shown). To further examine the

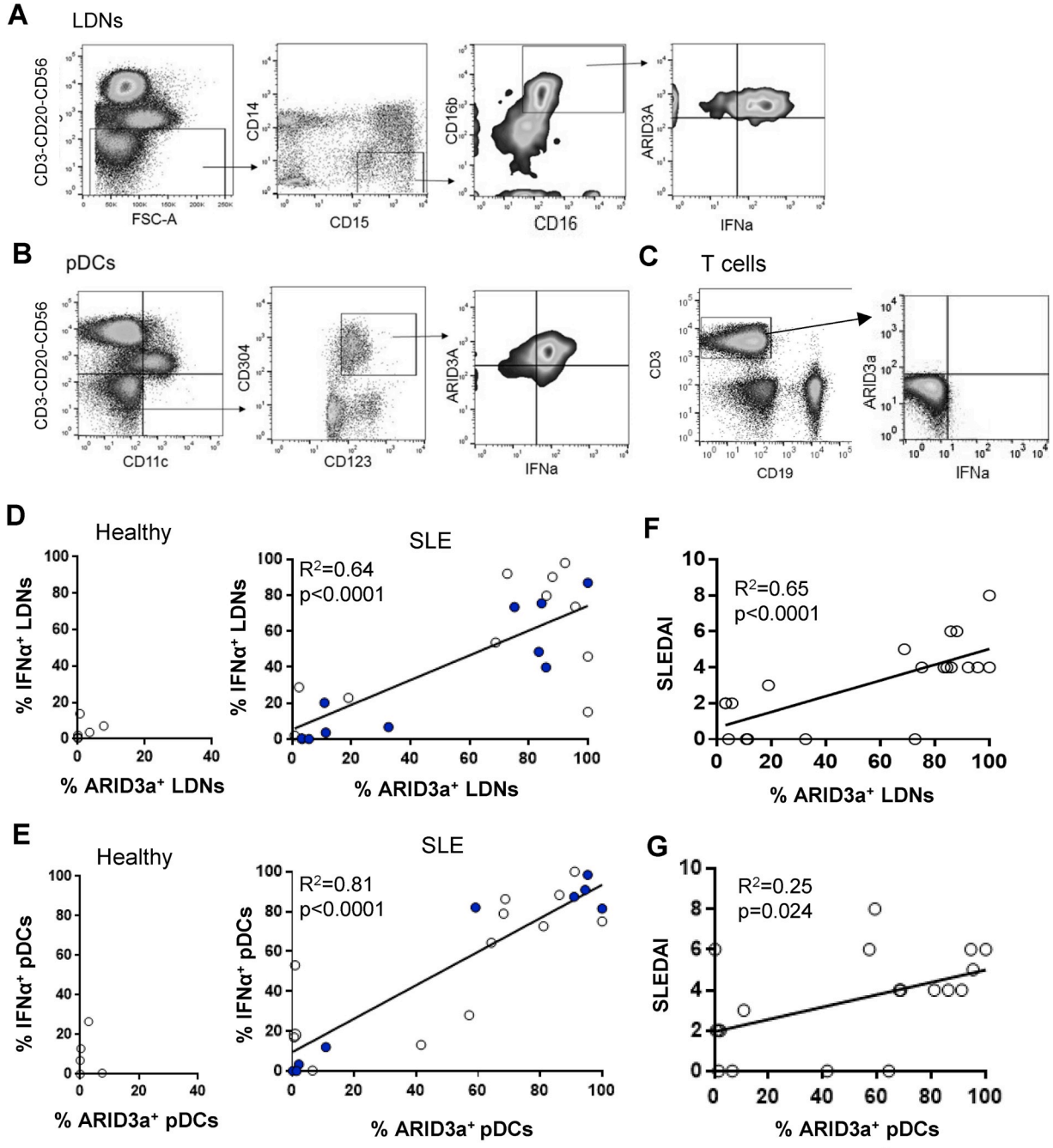

Fig. 1. ARID3a expression in SLE LDNs and pDCs is correlated with IFNα production, and with disease activity in LDNs. Flow cytometry of healthy control (n = 9) and SLE patient LDNs ($CD3^-CD20^-CD56^-CD14^-CD15^+CD16^+CD16b^+$) and pDCs ($CD3^-CD20^-CD56^-CD11c^-CD123^+CD304^+$) (n = 23) were evaluated for intracellular ARID3a and IFNα protein expression. Representative gating strategies for LDNs (A), pDCs (B) and internal control T cells (C) are shown. Associations between %$ARID3a^+$ and %$IFN\alpha^+$ LDNs (D) and pDCs (F) were analyzed by linear regression. Associations between patient SLEDAI scores at the time of blood draw and % $ARID3a^+$ LDNs (E) and pDCs (G) were determined by linear regression analyses. R^2 and p values are presented. Each point represents an individual patient sample. Solid points are samples used for later transcriptome analyses.

potential influence of numbers of ARID3a- or IFNα-producing cells on SLEDAI, we performed additive and subtractive step-wise multiple linear regression on all SLE samples using R. Multiple regression was performed independently for LDNs (n = 19) and pDCs (n = 20). SLEDAI was associated with % $ARID3a^+$ LDNs ($R^2 = 0.65$, $p = 0.00003$), while % $IFN\alpha^+$ LDNs did not contribute significantly to the predictive effect of the model. In contrast, while % $IFN\alpha^+$ pDCs contribute significantly to the model predicting SLEDAI ($R^2 = 0.29$, $p = 0.015$), % $ARID3a^+$ pDCs does not. Therefore, ARID3a was more strongly associated with disease activity in LDNs than in pDCs.

3.3. High ARID3a expression is associated with distinct gene profiles in LDNs and pDCs

To better understand how ARID3a, a transcription regulator, contributes to disease activity, RNA-seq analyses were performed on isolated samples of LDNs and pDCs from healthy controls and SLE patient samples with a wide range of ARID3a protein expression. ARID3a is an intracellular protein, so it is not possible to sort for $ARID3a^+$ cells without damaging RNA integrity. In addition, pDCs and LDNs are non-abundant subsets that are notoriously short-lived. To assess the

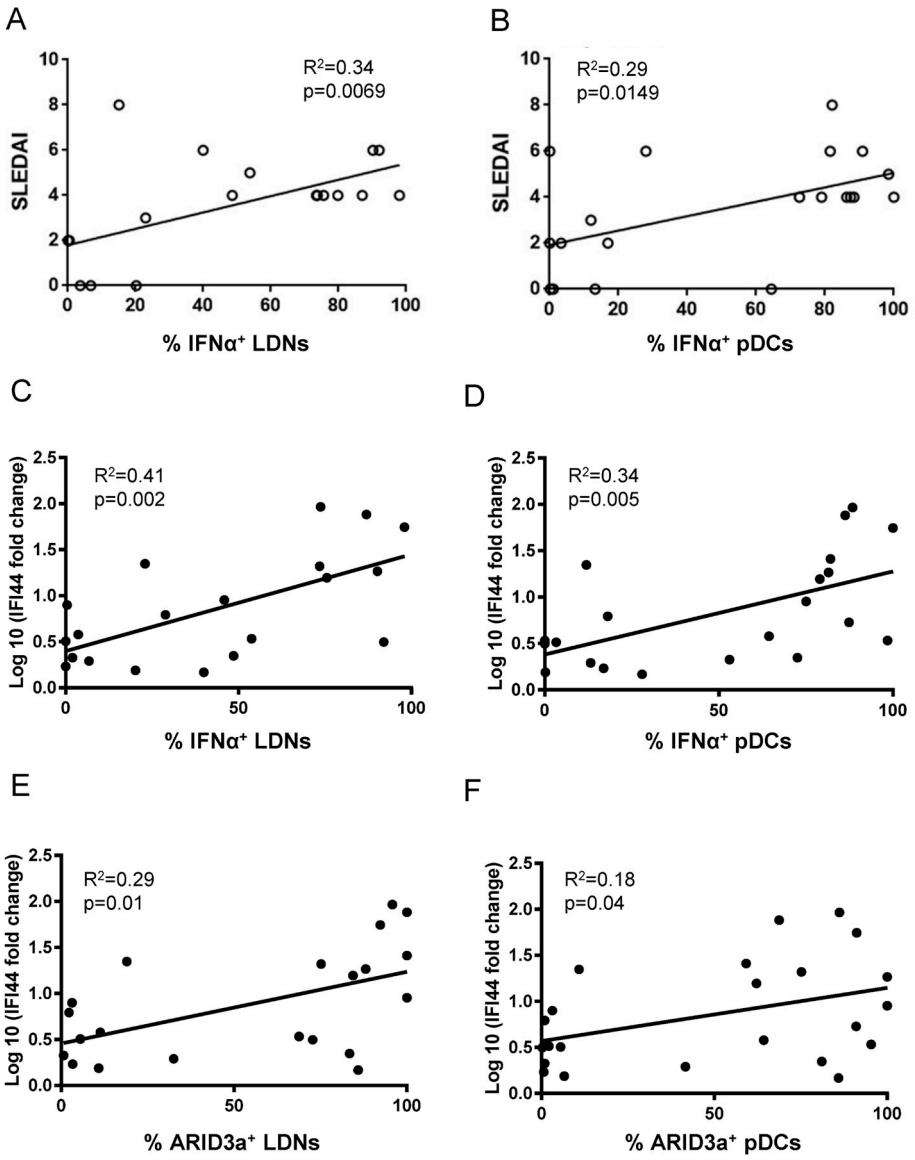

Fig. 2. IFNα only weakly associates with disease activity and ARID3a expression. SLE samples were analyzed for associations between SLEDAI scores and % IFNα$^+$ LDNs (A) and pDCs (B) via linear regression. Plasma IFNα activity, via induction of the IFNα signature gene *IFI44* was measured by qRT-PCR, and log-transformed values were tested for association with % IFNα$^+$ (C,D) and %ARID3a$^+$ (E,F) LDNs (C,E) and pDCs (D,F) via linear regression. R^2 and p values are given.

feasibility of performing transcriptome analyses with these cell types, we undertook a pilot study using cryopreserved PBMCs from 3 healthy samples, 3 SLE samples with low numbers of ARID3a$^+$ cells (< 40%, Fig. 1D, solid dots), and 3 SLE samples with high numbers of ARID3a$^+$ cells (> 70%). Samples that failed to meet library quality control criteria were eliminated from analyses. In both LDNs and pDCs, high-ARID3a SLE samples clustered together by gene expression, while low-ARID3a SLE samples clustered with healthy controls and were indistinguishable by gene expression profiles (Fig. 3A). Unlike our findings in B lymphocytes where *ARID3a* transcripts correlate closely with ARID3a protein levels [8,9], all LDN and pDC samples expressed *ARID3A* transcripts regardless of SLE or control status. These data were confirmed with freshly isolated cells from 2 healthy controls, and 2 each of high and low ARID3a-expressing SLE samples. As in cryopreserved samples, fresh SLE samples with low numbers of ARID3a-expressing cells clustered with healthy controls using unsupervised hierarchical clustering of gene expression patterns (Fig. 3B). These data suggest that ARID3a, as a transcription regulator, could directly or indirectly contribute to differences observed in gene expression patterns in SLE patients.

3.4. ARID3a and IFNα protein levels are associated with distinct gene profiles

Due to constitutive ARID3a transcript expression in LDNs and pDCs, determining how ARID3a is associated with changes in gene expression in Fig. 3 required analysis of data in association with ARID3a protein levels. Therefore, Spearman correlation analyses were performed to identify genes associated directly or indirectly with ARID3a or IFNα protein levels, as determined by flow cytometry (Fig. 4A). Variable total numbers of pDCs and LDNs are common in SLE blood samples [30,32,45], consistent with our findings (Fig. 4). Hierarchical clustering analyses were used to cluster the samples according to protein expression with transcriptome data from nine samples of SLE patient pDCs with ARID3a protein frequencies ranging from 100% to virtually undetectable levels. In pDCs, 592 genes were significantly correlated with ARID3a protein expression levels (Spearman's correlation, unadjusted, $p < 0.05$), while 680 genes were found to be correlated with IFNα protein levels in the same samples (unadjusted $p < 0.05$). Three examples of genes associated with % ARID3a$^+$ pDCs (Fig. 4B) have potential roles in gene regulation. In the pDCs, 189 genes were

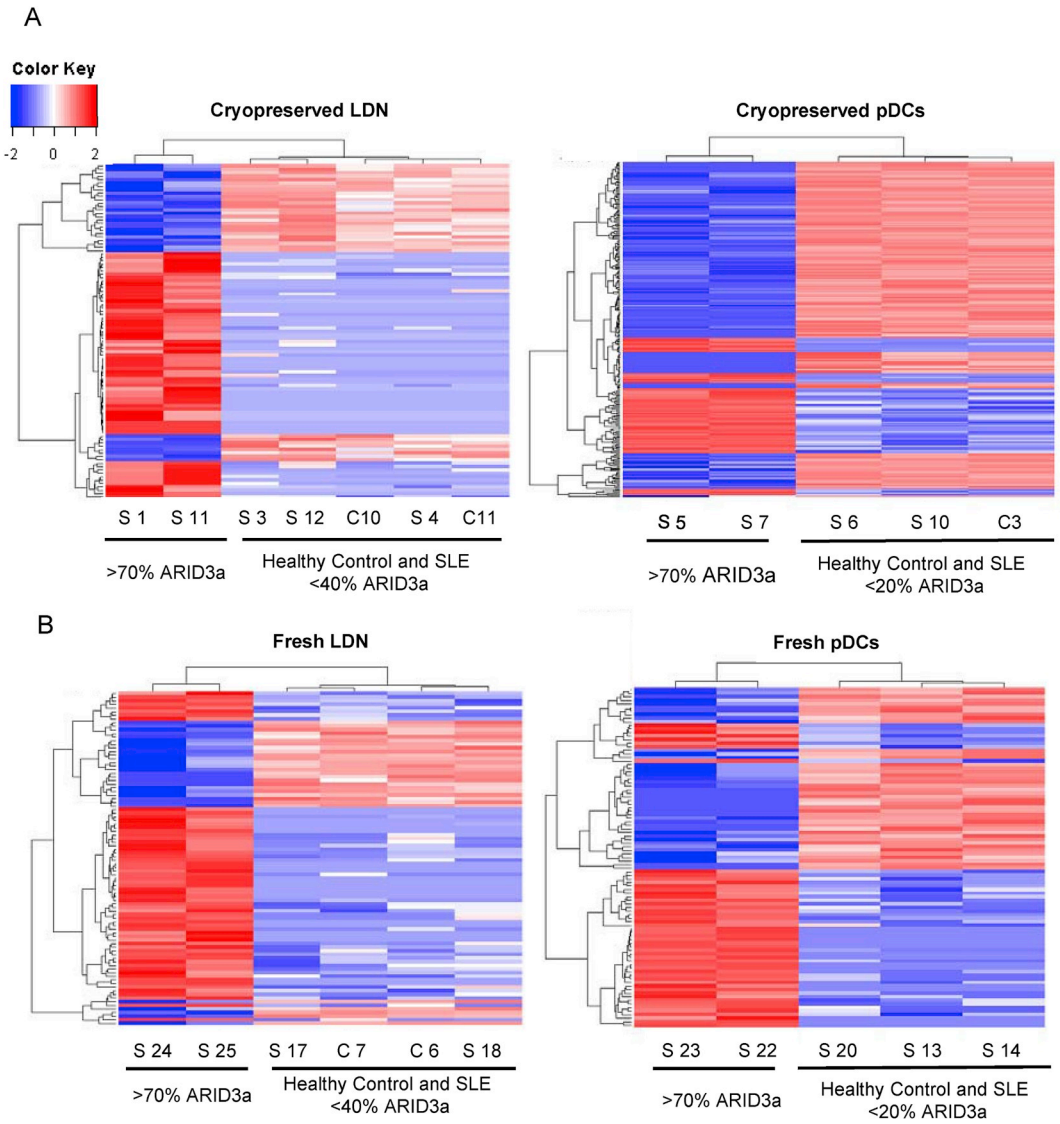

Fig. 3. Gene expression patterns of SLE LDN and pDCs samples with low ARID3a protein levels resemble healthy controls. Gene expression patterns obtained from RNA-seq of healthy control and SLE patient LDN and pDC samples with a wide range of ARID3a protein expression (solid points in Fig. 1D, E) were subjected to unsupervised hierarchical clustering of genes with FDR adjusted p values < 0.05. Heat maps of top differentially expressed genes from cryopreserved (A) and freshly isolated (B) LDNs and pDCs are shown. Patient (S) and control (C) samples are indicated with relative levels of ARID3a protein expression.

significantly associated with both ARID3a and IFNα protein expression, and heat maps representing the pairwise correlation coefficients (Spearman's correlation) among genes correlated with either ARID3a or IFNα expression are shown in Fig. 4C. These data reveal two tightly correlated clusters of genes that appear to be up- or down-regulated in tandem, suggesting the presence of genetic profiles tightly correlated with ARID3a protein expression. Similar analyses were performed using transcriptome data obtained from 10 samples of SLE patient LDNs that ranged in ARID3a protein expression levels from 100 to 3.2% (Fig. 4D). These data show 223 genes correlated with ARID3a protein expression in LDNs, while 1552 genes are correlated with IFNα protein expression in these same cells (unadjusted $p < 0.05$). Genes correlated with both ARID3a and IFNα protein expression (122 genes) are shown in association with ARID3a or IFNα in hierarchical clustered heat maps (Fig. 4E). Two major groups of up- or down-regulated genes are visible when plotted by association with ARID3a (left panel) or IFNa (right panel) for both pDCs and LDNs (Fig. 4C and E). Therefore, ARID3a may be linked to other master regulators that control expression of large groups of genes, or it may itself function as a master regulator.

3.5. A subset of genes are associated with both ARID3a and IFNα expression

Numbers of genes significantly associated with ARID3a and/or IFNα protein levels ($p < 0.05$) are presented as Venn diagrams for both pDCs (Fig. 5A) and LDNs (Fig. 5B). Overlap in the Venn diagrams showing genes associated with both ARID3a and IFNα protein expression are the genes depicted in the heat maps in Fig. 4C and E. Genes most strongly associated with both ARID3a and IFNα protein expression in pDCs with absolute r values of 0.7 or greater are listed (Fig. 5C). Similarly, genes with absolute r values of 0.6 or greater that correlated with both ARID3a and IFNα protein levels in LDNs are indicated (Fig. 5D). Gene Ontology enrichment analyses of gene sets correlated with both ARID3a and IFNα in both cell types are significantly enriched for sulfite oxidation and metabolic degradation pathways. The interferome webtool (http:interferome.org) also validated associations of these genes with interferon pathways. Genes associated with ARID3a expression alone in pDCs are enriched for IL17 antiviral responses. The genes associated only with ARID3a expression in LDNs are enriched for other transcription factors (31%), long non-coding RNAs and miRNAs, RNA-binding proteins and enzymes that modify chromatin. These data

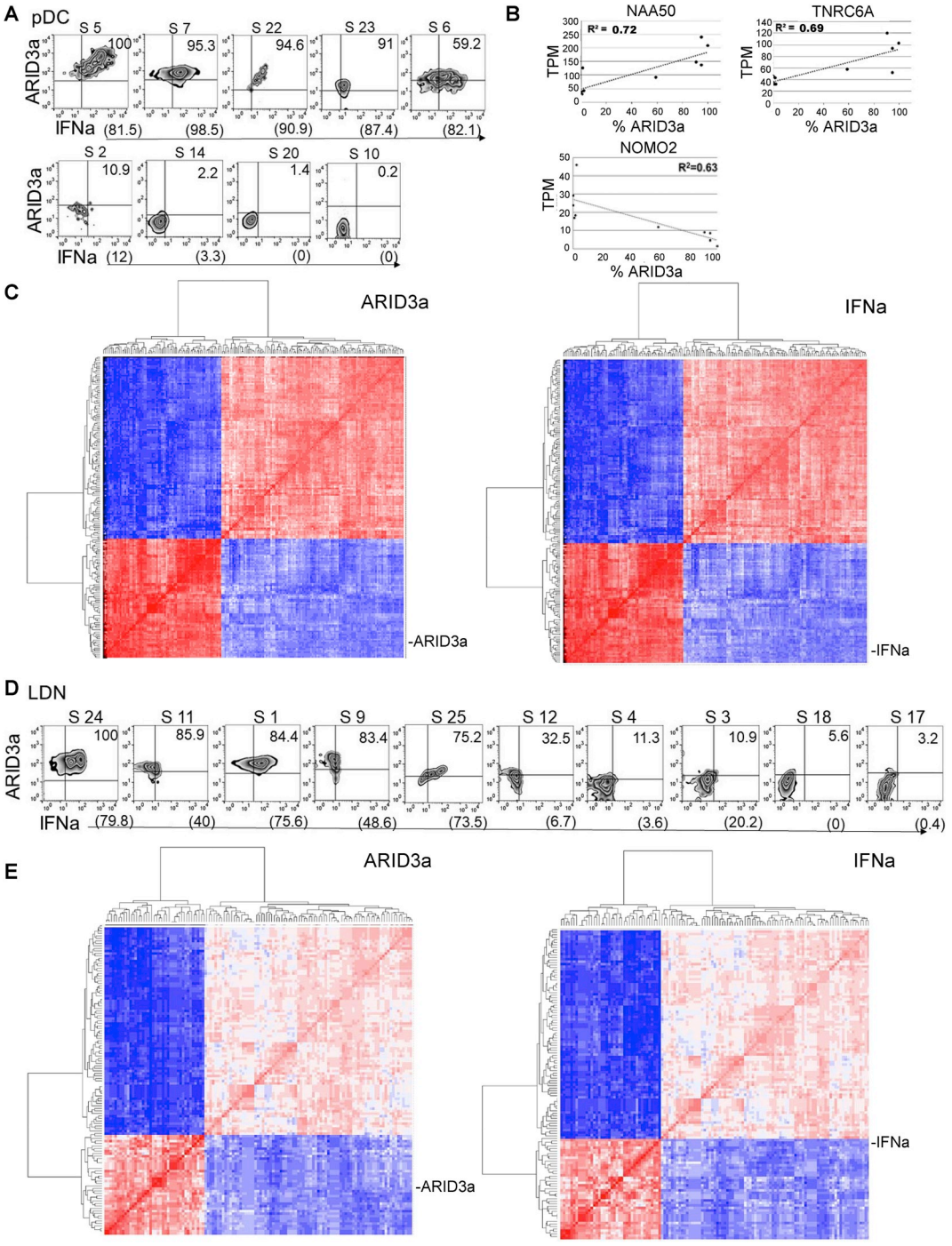

Fig. 4. Cells expressing both ARID3a and IFNα protein exhibit co-regulated gene profiles in pDCs and LDNs. Transcriptomes from 9 SLE pDC samples with varying levels of ARID3a and IFNα protein expression as shown by flow cytometry (A) were evaluated for associations between TPM and % ARID3a$^+$ or % IFNα$^+$ cells by Spearman's correlation. (B) Examples of linear regression analyses of 3 representative genes are shown with R^2. (C) Genes significantly ($p < 0.05$) correlated with expression of both ARID3a and IFNα in pDCs were clustered via heatmap with % ARID3a$^+$ pDCs by Spearman's correlation coefficient value (left panel); similarly, those genes were clustered with numbers of IFNα$^+$ pDCs (right panel). The analysis was repeated in LDNs (D, E). Color of cells indicates positive (red) or negative (blue) correlation coefficient of gene expression when compared to % ARID3a$^+$ or % IFNα$^+$ cells; intensity indicates strength of coefficient.

are consistent with ARID3a functions as a master transcription regulator.

4. Discussion

The ability to segregate SLE patients with high and low disease activity would be clinically beneficial. We found that the transcription regulator ARID3a is expressed in LDNs and pDCs, and that expression of ARID3a is associated with IFNα production in those cells. Surprisingly, high numbers of ARID3a-expressing LDNs are also associated with increased disease activity indices in SLE. Transcriptome analyses of patient samples with broad ranges of ARID3a expression revealed that samples with the highest percentage of ARID3a-expressing cells showed distinct gene profiles compared to SLE samples with low numbers of ARID3a-expressing cells which clustered with the healthy control samples for both pDCs and LDNs. Finally, gene expression profiles

A

B

C

pDCs

Gene	ARID3a r	Gene	ARID3a r	Gene	ARID3a r
VIPR1-AS1	-0.92	*GIN1*	-0.76	*LINC00702*	0.72
IL17RB	-0.91	*E2F1*	-0.76	*NF1*	0.72
THNSL1	-0.90	*FAM47E-STBD1*	-0.76	*TFDP2*	0.72
PCDHGB3	-0.89	*CACNB3*	-0.75	*RASAL2-AS1*	0.73
GDI2P2	-0.87	*FAH*	-0.75	*ATP6V1E1*	0.74
EID3	-0.87	*AVEN*	-0.75	*KIAA0100*	0.74
KIAA1614	-0.87	*IGKV6-21*	-0.75	*TOR1AIP1*	0.74
NCLP1	-0.87	*CTLA4*	-0.75	*TTC17*	0.74
FAM21FP	-0.87	*CHST15*	-0.74	*MGAT4EP*	0.74
NOMO2	-0.86	*TNS4*	-0.74	*FOXE1*	0.74
SUOX	-0.85	*CHCHD4*	-0.74	*AKT3*	0.74
H2AFY2	-0.85	*ST6GALNAC2*	-0.74	*PPP3CB-AS1*	0.74
WNT4	-0.85	*FAT2*	-0.73	*SRR*	0.74
C17orf77	-0.85	*ANK1*	-0.73	*ASB7*	0.75
AMN	-0.84	*CAGE1*	-0.73	*CAPZA2*	0.75
LCA5	-0.84	*EXOC3-AS1*	-0.73	*NEMP1*	0.75
SNORD97	-0.83	*OSGEPL1-AS1*	-0.72	*CCDC14*	0.76
WRB	-0.83	*PTPN13*	-0.72	*PWWP2A*	0.76
PREX2	-0.82	*POTEC*	-0.72	*TSC22D2*	0.76
TRIB1	-0.82	*ATRN*	-0.72	*PIP5KL1*	0.76
NUP62CL	-0.82	*CAD*	-0.72	*ZNF99*	0.77
ZMYND15	-0.82	*TLDC1*	-0.72	*GPR141*	0.77
SYNGAP1	-0.82	*MARCKSL1P1*	-0.72	*HIVEP3*	0.77
OXCT2	-0.81	*ENAM*	-0.71	*PREPL*	0.77
HIPK1-AS1	-0.81	*TTC30B*	-0.71	*NTRK2*	0.78
GGT7	-0.81	*C16orf86*	-0.71	*DDHD2*	0.79
TMEM132A	-0.80	*IQCE*	-0.71	*CCL2*	0.80
PCDHGB5	-0.80	*KLRG1*	-0.71	*CCDC114*	0.81
CDC20	-0.79	*NAB2*	-0.71	*TC2N*	0.81
RPS17P13	-0.79	*CYP2D6*	-0.71	*GTF2E2*	0.82
STARD7-AS1	-0.79	*SDC1*	-0.70	*TMEM189*	0.82
PIPSL	-0.78	*LINC00680*	-0.70	*EIF4ENIF1*	0.82
AGFG2	-0.78			*TRAF6*	0.82
MELK	-0.78	*CRYZL1*	0.70	*CCNE2*	0.83
GATA6	-0.78	*FAM91A1*	0.70	*HDX*	0.83
TROAP	-0.77	*TNRC6A*	0.70	*IPO9*	0.83
C10orf128	-0.77	*PRLR*	0.71	*OR51E2*	0.84
MIR600HG	-0.77	*ANAPC13*	0.71	*DGKH*	0.85
NBPF13P	-0.76	*ATP6V1D*	0.71	*NHSL2*	0.85
CAPN10-AS1	-0.76	*WDR45B*	0.71	*NAA50*	0.88
C4A-AS1	-0.76	*RBMS3-AS2*	0.71	*SFXN1*	0.89
C4B-AS1	-0.76	*AC005562.1*	0.71	*ZC3H8*	0.89
CDK8	-0.76	*RPS4XP14*	0.72		

D

LDN

Gene	ARID3a r
CLLU1	-0.87
DDAH1	-0.75
MAP3K7CL	-0.74
BMS1P2	-0.74
XCL2	-0.70
RBM43	-0.70
TJP2	-0.68
SLAMF9	-0.67
MRPL35	-0.66
CORO7-PAM16	-0.66
FAM81A	-0.65
HCG25	-0.65
CYP1B1	-0.64
RPS10-NUDT3	-0.63
LPCAT2	-0.63
DGKZ	-0.63
GCSAML	-0.62
CXCL17	-0.61
FOLH1	0.60
CFAP57	0.60
TMEM191B	0.61
CPLX3	0.61
ZFP91-CNTF	0.61
GAP43	0.61
GOLGA8F	0.62
CAPN11	0.63
CYP4B1	0.63
FAM167A	0.64
CLDN4	0.66
NPM1P12	0.66
NPTX1	0.66
RPL36AP33	0.68
LAMC2	0.68
ITGA11	0.69
TGIF2P1	0.71
C6orf10	0.72
AC105342.1	0.72
AC012066.1	0.72
PRRX1	0.73
LINC00487	0.75
ERICH3	0.81

Fig. 5. Distinct gene subsets are associated with both ARID3a and IFNα protein in pDCs and LDNs. Venn diagrams depict genes associated with ARID3a and IFNα protein levels in pDCs (A) and LDNs (B). Genes that correlated with both ARID3a and IFNa protein expression in the Venn diagrams above with absolute ARID3a associated r values > 0.7 in pDCs (C) and with absolute ARID3a-associated r values > 0.6 in LDNs (D) are listed.

suggest that ARID3a is a regulator of inflammatory pathways involved in SLE.

While increased plasma IFNα has been associated with increased disease activity [12–16], others noted that IFNα gene signatures were not associated with longitudinal changes in disease activity [46]. Similarly, we found weak correlations between IFNα-expression and increased disease activity in both LDNs and pDCs, and were surprised that ARID3a expression is more highly associated with disease activity in LDNs. Our data suggest that IFNα may also contribute to disease activity, but are consistent with data suggesting that factors other than IFNα production contribute to SLEDAI scores in SLE [30,31,47,48].

Our data identify genes associated with both ARID3a and IFNα protein expression. Interestingly, the large majority of genes that are correlated with ARID3a and/or IFNa production differ between pDCs and LDNs, indicating that ARID3a expression identifies gene profiles that are cell type-specific. The large numbers of genes that are coordinately up- and/or down-regulated as a group in association with ARID3a expression in both pDCs and LDNs suggest that ARID3a may act as a master gene regulator, or that it is closely associated with other master gene regulators. In LDNs, thirty-one percent of the genes associated with variation in ARID3a protein expression are transcription factors, including *Sox2* and *Nanog*. Other ARID family proteins affect large numbers of genes epigenetically, [reviewed in ref. [49,50]]. In single cell analyses of the K562 cell line, ARID3a is associated with distinct regulatory states and chromatin configurations [51]. More recently, ARID3a was shown to bind near the edges of enhancer regions in

many cell types [52], suggesting it may also function epigenetically.

We did not observe upregulation of any of the 13 *IFNA* subtypes in our analyses, despite the presence of IFNα protein in both LDNs and pDCs. The levels of individual *IFNA* transcripts may be below the level of detection using RNA-seq technology. *IFNA* detection typically requires quantitative PCR analyses. Therefore, RNA-seq data have limitations that may preclude detection of all transcripts differentially expressed in association with ARID3a protein expression. Another limitation of RNA-seq data highlighted by our study is that RNA levels do not always correlate with protein levels. Others demonstrated that ARID3a is regulated by miRNAs in early hematopoiesis [53,54]. These studies suggest that regulation of ARID3a by miRNAs is cell type-specific. We speculate that miRNA regulation of ARID3a also occurs in pDCs and LDNs, and that miRNA control of protein expression would allow rapid responses to extracellular signals potentially explaining the discrepancy between *ARID3A* transcripts and protein levels in these cells. Our RNA-seq data did not detect the miR125b and lin28b miRNAs associated with ARID3a regulation in early B lymphocyte progenitors. Further experiments will be required to determine if other differentially expressed miRNAs with potential ARID3a target sites may function in neutrophils and pDCs.

Our data implicate ARID3a as an important regulator of inflammatory responses and IFNα production in several cell types, and identify gene profiles associated with ARID3a expression in pDCs and LDNs from SLE patients. Most surprising was the finding that increased disease activity in SLE is more strongly associated with frequencies of ARID3a$^+$ LDNs than with IFNα$^+$ cells. While a major function of pDCs is cytokine secretion, LDNs also undergo NETosis [30,31]. Therefore, ARID3a may regulate genes important for disease activity, but that are distinct from IFNα regulatory pathways.

We do not know whether ARID3a regulates IFN production, or if IFNα production stimulates ARID3a expression in pDCs and LDNs. However, in B lymphocytes, developmentally less mature cells expressed ARID3a without IFNα, and induction of ARID3a transcripts occurred prior to detection of IFNα transcripts [8]. Furthermore, inhibition of ARID3a in a B cell line also inhibited IFNα expression [8]. Regardless of which protein is upstream in pDCs and LDNs, a large number of genes appear to be co-regulated in correlation with ARID3a and IFNα. These data strongly suggest that ARID3a is associated with other master regulators of gene expression, and that it could be mechanistically involved in innate immune responses in SLE. We speculate that ARID3a and its gene targets may offer new therapeutic approaches.

Disclosure statement

The authors declare no competing financial interests.

Author contributions

CFW – conceptualized studies; MLR, CFW- designed experiments; MLR, JG, CFW – wrote manuscript; MLR, JG, MDB – performed research; MLR, JG, LG, CM, CG, KW, CFW – analyzed data; MLR, JG, LG, CM, JW, JAJ, CFW – interpreted data; LG, CM, MDB, EC, JAJ – edited manuscript; EC, JAJ-provided human samples; EC- characterized patients.

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Appendix A. Supplementary data

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