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Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naïve CD4+ T cells from lupus patients

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ABSTRACT

Systemic lupus erythematosus is an autoimmune disease characterized by multi-system involvement and autoantibody production. Abnormal T cell DNA methylation and type-I interferon play an important role in the pathogenesis of lupus. We performed a genome-wide DNA methylation study in two independent sets of lupus patients and matched healthy controls to characterize the DNA methylome in naïve CD4+ T cells in lupus. DNA methylation was quantified for over 485,000 methylation sites across the genome, and differentially methylated sites between lupus patients and controls were identified and then independently replicated. Gene expression analysis was also performed from the same cells to investigate the relationship between the DNA methylation changes observed and mRNA expression levels. We identified and replicated 86 differentially methylated CG sites between patients and controls in 47 genes, with the majority being hypomethylated. We observed significant hypomethylation in interferon-regulated genes in naïve CD4+ T cells from lupus patients, including *IFIT1*, *IFIT3*, *MX1*, *STAT1*, *IFI44L*, *USP18*, *TRIM22* and *BST2*, suggesting epigenetic transcriptional accessibility in these genetic loci. Indeed, the majority of the hypomethylated genes (21 out of 35 hypomethylated genes) are regulated by type I interferon. The hypomethylation in interferon-regulated genes was not related to lupus disease activity. Gene expression analysis showed overexpression of these genes in total but not naïve CD4+ T cells from lupus patients. Our data suggest epigenetic "poising" of interferon-regulated genes in lupus naïve CD4+ T cells, argue for a novel pathogenic implication for abnormal T cell DNA methylation in lupus, and suggest a mechanism for type-I interferon hyper-responsiveness in lupus T cells.

1. Introduction

Systemic lupus erythematosus is a chronic autoimmune disease characterized by the production of antinuclear antibody and multiple organ involvement. The etiology of lupus is incompletely understood, but clear evidence suggests an important role for abnormal T cell DNA methylation in the pathogenesis of the disease [1]. Indeed, demethylated T cells are sufficient to cause a lupus-like disease in mouse models [2].

DNA methylation is an epigenetic mechanism that regulates gene expression by altering transcriptional accessibility of regulatory regions within gene sequences. This chemical modification of cytosine residues most commonly occurs in CG dinucleotides, and is mediated by DNA methyltransferase enzymes [3]. In general, methylation of CG dinucleotides in regulatory sequences induces gene silencing, while hypomethylation allows for transcriptional chromatin accessibility, and active gene expression when appropriate transcription factors are available [3]. DNA methylation induces chromatin inaccessibility by several mechanisms, including recruitment of histone deacetylases that remove acetyl groups from histone tails thereby increasing the charge attraction between DNA and histone proteins to generate more compact chromatin configuration that prevents access by the transcriptional machinery [4].

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DNA methylation plays an important role in T cell differentiation. Indeed, the interferon gamma locus demethylates upon T_H1 differentiation, and the interleukin (IL)-4, IL-5, and IL-13 common locus control region demethylates upon T_H2 differentiation, allowing for interferon gamma, and IL-4, IL-5, and IL-13 production in differentiated T_H1 and T_H2 cells, respectively [5]. In contrast, both loci are heavily methylated in naïve CD4+ T cells [5].

We have previously characterized DNA methylation changes in total CD4+ T cells from lupus patients and revealed wide-spread DNA methylation changes in patients compared to healthy controls [6]. Herein, we performed an extensive genome-wide DNA methylation study in naïve CD4+ T cells from lupus patients and controls, coupled with gene expression profiling from the same cells. We identified DNA methylation changes prior to T cell differentiation and activation in lupus and determined the effect of these methylation changes on gene expression.

2. Methods

2.1. Lupus patients and controls

We studied two independent sets of female lupus patients and controls, each consisting of 36 participants (18 lupus patients and 18 healthy controls). We designed our study to include a discovery cohort and a second independent cohort for replication. The discovery cohort was recruited from the Oklahoma Lupus Cohort at the Oklahoma Medical Research Foundation (OMRF), and the replication cohort was subsequently recruited from the University of Michigan rheumatology clinics. Patients and controls in both sets were matched for age (+/−5 years) and ethnicity (Table 1). Our study was approved by the institutional review boards at OMRF and the University of Michigan. All study participants singed a written informed consent prior to participation in the study.

2.2. Naïve CD4+ T cell isolation and purity

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood samples obtained from patients and controls using density gradient centrifugation (Amersham Biosciences, Uppsala, Sweden). Naïve CD4+ T cells were separated from PBMCs using the Naive CD4+ T Cell Isolation Kit II (Miltenyi Biotec, Cambridge, MA) which allows for the indirect isolation of untouched naïve CD4+ T cells. Naïve CD4+ T cell purity was confirmed by flow cytometry using fluorochrome-conjugated antibodies against CD4 and CD45RA; we have consistently achieved cell purity of >95% (Supplementary Fig. 1). DNA was isolated using the DNeasy Kit (Qiagen, Valencia, CA) for subsequent use in the DNA methylation studies. An aliquot of naïve CD4+ T cells from a subset of the samples was stored in TRIzol® reagent at −80 °C and later used for RNA extraction and mRNA expression experiments.

2.3. DNA methylation studies and array validation

Genome-wide DNA methylation in naïve CD4+ T cells from lupus patients and controls included in the discovery and the replication cohorts was assessed using the Illumina Infinium HumanMethylation450 BeadChip array. This array allows for the interrogation of over 485,000 methylation sites within the entire genome. This array covers 99% of RefSeq genes, with an average of 17 CG sites per gene across the promoter region, 5′-UTR, first exon, gene body, and 3′-UTR. It also covers 96% of CG islands. Non CG methylation sites recently identified in human stem cells are also covered as well as microRNA promoter regions. Validation of the array data was performed using bisulfite DNA sequencing in known hypermethylated and hypomethylated regions as previously described [6].

2.4. Gene expression studies

RNA extraction was performed using a combination of TRIzol (Invitrogen, Carlsbad, CA) and RNeasy kits (Qiagen, Valencia, CA) as previously described [7]. RNA concentration was determined with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and then qualitatively assessed for degradation with 28:18S ribosomal RNA, using a capillary gel electrophoresis system (Agilent 2100 Bioanalyzer, Agilent, Wilmington, DE). Gene expression profiling was performed in naïve CD4+ T cells from lupus patients and controls using the HumanHT-12 v4 Expression BeadChip array (Illumina).

2.5. Statistical and bioinformatics analysis

DNA methylation analysis was performed using GenomeStudio methylation analysis package (Illumina) as previously described [6].

Table 1
Demographic characteristics and disease activity as measured by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores in the lupus patients included in our study. Two independent cohorts of lupus patients and age-, sex- and ethnicity-matched controls were included in this study.

Cohort 1									Cohort 2								
Patients					Controls				Patients					Controls			
Number	Age	Sex	Ethnicity	SLEDAI	Number	Age	Sex	Ethnicity	Number	Age	Sex	Ethnicity	SLEDAI	Number	Age	Sex	Ethnicity
1	33	Female	EA	6	C1	35	Female	EA	19	22	Female	AA	6	C19	25	Female	AA
2	58	Female	EA	0	C2	53	Female	EA	20	51	Female	EA	3	C20	47	Female	EA
3	33	Female	EA	4	C3	30	Female	EA	21	58	Female	EA	6	C21	55	Female	EA
4	50	Female	AA	4	C4	52	Female	AA	22	54	Female	EA	10	C22	50	Female	EA
5	36	Female	EA	0	C5	41	Female	EA	23	25	Female	EA	4	C23	24	Female	EA
6	25	Female	EA	2	C6	26	Female	EA	24	18	Female	EA	4	C24	23	Female	EA
7	58	Female	EA	2	C7	58	Female	EA	25	36	Female	EA	2	C25	36	Female	EA
8	35	Female	AA	0	C8	35	Female	AA	26	40	Female	EA	2	C26	45	Female	EA
9	30	Female	EA	2	C9	27	Female	EA	27	34	Female	AA	4	C27	32	Female	AA
10	30	Female	AA	4	C10	32	Female	AA	28	41	Female	EA	6	C28	38	Female	EA
11	40	Female	EA	4	C11	40	Female	EA	29	63	Female	EA	0	C29	58	Female	EA
12	57	Female	AA	2	C12	57	Female	AA	30	38	Female	EA	12	C30	36	Female	EA
13	41	Female	EA	4	C13	45	Female	EA	31	39	Female	EA	2	C31	41	Female	EA
14	29	Female	EA	2	C14	34	Female	EA	32	53	Female	EA	2	C32	53	Female	EA
15	25	Female	AA	0	C15	27	Female	AA	33	24	Female	EA	4	C33	26	Female	EA
16	26	Female	Asian	4	C16	29	Female	Asian	34	33	Female	AA	4	C34	34	Female	AA
17	66	Female	EA	0	C17	64	Female	EA	35	40	Female	EA	6	C35	40	Female	EA
18	34	Female	EA	2	C18	30	Female	EA	36	34	Female	AA	2	C36	38	Female	AA

SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; EA, European-American; AA, African-American.