ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201806.00194

Abnormal H3K27me3 Modification in Promoter Regions Promotes CREM Overexpression in CD4+ T Cells of Systemic Lupus Erythematosus Patients

Authors: Zhang Qing, Ding Shu, Huilin Zhang

Date: 2018-06-15T00:00:00+00:00

Abstract

Objective: To investigate the cause of elevated CREM expression in SLE. Methods: CD4+ T cells were isolated from 5 healthy controls and 5 SLE patients, and chromatin immunoprecipitation (ChIP) microarray was used to analyze the levels of histone H3 lysine 27 trimethylation (H3K27me3) in the promoter regions of various genes. Subsequently, CD4+ T cells were isolated from 30 healthy controls and 30 SLE patients, and ChIP combined with real-time quantitative PCR was used to detect the levels of H3K27me3, H3K27 demethylases JMJD3 and UTX, and H3K27 methyltransferase EZH2 in the CREM promoter region, while real-time quantitative RT-PCR was used to detect CREM mRNA levels. Results: The level of H3K27me3 in the CREM promoter region of SLE CD4+ T cells was 0.23 times that of healthy controls. Subsequently, through ChIP combined with real-time quantitative PCR, we confirmed that the level of H3K27me3 in the CREM promoter region of SLE patients' CD4+ T cells was significantly decreased ($P<0.001$), and was significantly negatively correlated with CREM mRNA levels ($P<0.001$). The level of JMJD3 in this region was significantly increased ($P<0.001$), and was negatively correlated with H3K27me3 levels ($P<0.001$) and positively correlated with CREM mRNA levels ($P<0.001$). However, the levels of UTX ($P=0.172$) and EZH2 ($P=0.281$) showed no significant difference compared with the control group. Conclusion: Increased JMJD3 in the CREM promoter region of SLE CD4+ T cells leads to decreased H3K27me3 levels in this region, resulting in CREM overexpression and ultimately contributing to the pathogenesis of SLE.

ChinaRxiv 中X

Full Text

Effect of Aberrant H3K27me3 Modification in Promoter Regions on cAMP Response Element Modulator Expression in CD4+ T Cells from Patients with Systemic Lupus Erythematosus

Qing Zhang[1], Shu Ding[2], Huilin Zhang[3]
[1]Department of Dermatology, [3]Department of Emergency Medicine, Second Xiangya Hospital, Central South University, Changsha 410011, China;
[2]Department of Dermatology, Third Xiangya Hospital, Central South University, Changsha 410013, China

Abstract

Objective: Increased cAMP response element modulator (CREM) in T cells plays an essential role in the pathogenesis of systemic lupus erythematosus (SLE). The aim of this study was to investigate the mechanisms that elevate CREM expression in SLE.

Methods: CD4+ T cells from five healthy volunteers and five SLE patients were isolated for analysis of histone H3 lysine 27 trimethylation (H3K27me3) levels in various gene promoters using chromatin immunoprecipitation (ChIP) microarray. Subsequently, CD4+ T cells from 30 normal controls and 30 SLE patients were isolated to measure levels of H3K27me3, the H3K27 demethylases JMJD3 and UTX, and the H3K27 methyltransferase EZH2 within the CREM promoter by ChIP combined with real-time quantitative PCR; CREM mRNA levels were also determined by real-time RT-PCR.

Results: Analysis of ChIP microarray data identified that H3K27me3 enrichment at the CREM promoter in CD4+ T cells from SLE patients was 0.23 times that of normal control subjects. ChIP and real-time PCR confirmed a marked decrease in H3K27me3 enrichment at the CREM promoter in SLE patients' CD4+ T cells ($P<0.001$), which was significantly negatively correlated with CREM mRNA levels ($P<0.001$). JMJD3 binding at this region was significantly elevated ($P<0.001$) and negatively correlated with H3K27me3 levels ($P<0.001$) while positively correlated with CREM mRNA levels ($P<0.001$). However, UTX ($P=0.172$) and EZH2 ($P=0.281$) levels showed no significant differences compared with controls.

Conclusion: Increased JMJD3 binding at the CREM promoter in SLE CD4+ T cells downregulates H3K27me3 enrichment, leading to CREM overexpression that may contribute to SLE pathogenesis.

Keywords: systemic lupus erythematosus; cAMP response element modulator ; CD4+ T cells; H3K27me3; JMJD3

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease involving multiple pathogenic mechanisms. In recent years, accumulating evidence has demonstrated that epigenetic alterations of specific genes in T cells play critical roles in SLE pathogenesis. Epigenetics refers to stable and heritable changes in gene expression without alterations in DNA sequence, primarily including DNA methylation, histone modifications, non-coding RNA regulation, and chromatin remodeling. Among these mechanisms, histone H3 lysine 27 trimethylation (H3K27me3), a hallmark of gene silencing, has attracted considerable attention. The level of H3K27me3 is regulated by the coordinated actions of histone demethylases JMJD3 and UTX, as well as the histone methyltransferase EZH2.

Studies have identified cAMP response element modulator (CREM) as a key factor in SLE pathogenesis. CREM levels are significantly elevated in T cells from SLE patients, and CREM promoter activity positively correlates with SLE disease activity index (SLEDAI). Elevated CREM contributes to SLE development through multiple mechanisms: First, increased CREM reduces IL-2 production, leading to impaired cytotoxic responses, decreased Treg cell numbers and function, and defective activation-induced cell death (AICD). Second, elevated CREM increases IL-17A, which interacts with various chemokines and cytokines to trigger multiple inflammatory responses. IL-17A also stimulates B cell proliferation, resulting in increased autoantibody production. Additionally, CREM overexpression inhibits transcription of the TCR/CD3 chain, preventing termination of T cell responses and causing persistent T cell activation. CREM also participates in SLE pathogenesis by suppressing transcription factor c-fos, antigen-presenting cell molecule CD86, and Notch signaling pathway molecule Notch-1. However, the molecular mechanisms underlying elevated CREM levels in SLE T cells remain unclear.

Through chromatin immunoprecipitation (ChIP) microarray analysis, we found that H3K27me3 levels at the CREM promoter in SLE patients' CD4+ T cells were significantly lower than in normal controls. Based on this clue, we further investigated the cause of elevated CREM expression in SLE CD4+ T cells to provide new insights into SLE pathogenesis.

Methods

1.1 Study Subjects

Thirty SLE patients were recruited from the outpatient clinic and inpatient department of the Department of Dermatology, Second Xiangya Hospital, Central South University. All patients fulfilled the 1997 American College of Rheumatology revised criteria for SLE classification. Clinical information for SLE patients is summarized in . The cohort included 27 females and 3 males, aged 20–42 years (28.567 ± 6.558), with SLEDAI scores ranging from 0–16 (7.567 ± 4.384). Thirty healthy controls were recruited from hospital staff and graduate students at Second Xiangya Hospital, including 27 females and 3 males aged 20–41 years

(27.133 ± 6.067). No statistically significant differences in age or gender were observed between patients and controls (P>0.05). All participants provided informed consent, and the study was approved by the Ethics Committee of Second Xiangya Hospital, Central South University.

1.2 Materials and Reagents

Lymphocyte separation medium was purchased from GE Healthcare (Sweden). CD4+ T cell positive selection kits were obtained from Miltenyi Biotec (Germany). ChIP assay kits were from Millipore (USA). TRIzol reagent was from Invitrogen (USA). SYBR® Premix Ex Taq™ (Tli RNaseH Plus) and One Step SYBR® PrimeScript™ RT-PCR kits were from Takara (Japan). Anti-H3K27me3 antibody was from Millipore (USA). Anti-JMJD3, anti-UTX, and anti-EZHZ antibodies were from Abcam (USA). PCR primers were synthesized by Shanghai Biosun Biotech.

1.3 Cell Isolation

Peripheral venous blood (60 mL) was collected from each subject with heparin anticoagulation (20 U/mL). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using lymphocyte separation medium. PBMCs were washed with PBS, and CD4+ T cells were positively selected using immunomagnetic beads.

1.4 ChIP Microarray

CD4+ T cells from five SLE patients and five age- and gender-matched healthy controls were fixed with 1% formaldehyde and lysed using lysis buffer. Lysates from SLE patients and controls were pooled separately and sent to CapitalBio Corporation (Beijing) for quality control, labeling, hybridization, scanning, and statistical analysis. DNA precipitated by anti-H3K27me3 antibody and total DNA (input) were labeled with Cy5 (red) and Cy3 (green), respectively. Samples were hybridized to microarray chips, and Cy3/Cy5 ratio images were generated, with color intensity representing relative H3K27me3 levels at various gene promoters. A greater than 2-fold increase or less than 0.5-fold decrease in H3K27me3 levels at gene promoters in SLE CD4+ T cells compared with normal controls was considered significant.

1.5 ChIP Combined with Real-Time Quantitative PCR

ChIP assays were performed using a ChIP assay kit according to the manufacturer' s instructions. Briefly, CD4+ T cells were fixed with 1% formaldehyde for 10 minutes and lysed using lysis buffer. DNA in the cell lysates was sheared by sonication, and supernatants were collected after centrifugation. Non-specific background was removed using protein G agarose beads, followed by overnight incubation with antibodies at 4°C with rotation. The next day, protein G agarose beads were added and incubated for 1 hour at 4°C

with rotation to bind immune complexes. After washing agarose bead-DNA-protein complexes, DNA-protein complexes were eluted using elution buffer and heated at 65°C for 4 hours to reverse cross-linking, after which DNA was purified. Real-time quantitative PCR was performed using SYBR® Premix Ex Taq™ (Tli RNaseH Plus) with a standard curve relative quantification method. Briefly, purified DNA was amplified as template, while a separate DNA sample was serially diluted (2, 4, 8, 16, and 32-fold) to generate a standard curve for calculating relative concentrations of each sample, with input DNA serving as internal reference. The fold enrichment of target protein-bound DNA relative to input DNA represented the relative quantification result. All experiments were performed in triplicate. Primer sequences were: CREM promoter forward 5' -TGGGGAGATAGAGGTTGCAG-3' , reverse 5' -CGCCAGAAATCCAATGACTT-3' . Cycling conditions were: 95°C for 15 seconds, 60°C for 20 seconds, 72°C for 30 seconds; followed by 95°C for 10 seconds, 60°C for 20 seconds, for 40 cycles.

1.6 RNA Extraction and Real-Time Quantitative One-Step RT-PCR

Total RNA from isolated CD4+ T cells was extracted using TRIzol according to the manufacturer' s instructions. RNA concentration and A260/A280 ratio were determined using a UV/visible spectrophotometer. All samples had A260/A280 ratios between 1.8-2.0. Extracted RNA was aliquoted and stored at -80°C. Real-time quantitative one-step RT-PCR was performed using the One Step SYBR® PrimeScript™ RT-PCR kit with a standard curve relative quantification method to detect mRNA levels. Similar to the real-time PCR method described above, CD4+ T cell RNA served as template with -actin amplified as internal reference. The fold change of target gene concentration relative to -actin represented the relative quantification result. All experiments were performed in triplicate. Primer sequences were: CREM forward 5' -GAAACAGTTGAATCCCAGCATGATGGAAGT-3' , reverse 5' -TGCCCCGTGCTAGTCTGATATATG-3' ; -actin forward 5' -CGCGAGAAGATTGACCCAGAT-3', reverse 5'-GCACTGTGTTGGCGTACAGG-3' . Cycling conditions were: 42°C for 5 minutes, 95°C for 10 seconds; followed by 95°C for 10 seconds, 60°C for 20 seconds, for 40 cycles.

1.7 Statistical Analysis

Data were stored and analyzed using SPSS 16.0 for Windows. Continuous variables were expressed as mean ± standard deviation. Comparisons between two independent groups were performed using two-sample t-tests. Pearson correlation coefficients were calculated for single-factor linear correlation analysis between experimental parameters. $P<0.05$ was considered statistically significant.

Results

2.1 ChIP Microarray Results

The ChIP microarray screened 20,832 gene promoters, among which 552 showed greater than 2-fold differences in H3K27me3 levels between groups. Notably, H3K27me3 enrichment at the CREM promoter in SLE CD4+ T cells was 0.23-fold that of normal control CD4+ T cells.

2.2 Validation of ChIP Microarray Results

To confirm the microarray findings, we used ChIP combined with real-time quantitative PCR to examine H3K27me3 levels at the CREM promoter in CD4+ T cells from 30 healthy controls and 30 SLE patients. Compared with normal controls, SLE patients showed significantly reduced H3K27me3 enrichment at the CREM promoter (normal control vs. SLE: 2.723 ± 0.659 vs. 0.489 ± 0.146, $P<0.001$), consistent with our ChIP microarray results. We further examined CREM mRNA levels and found a significant negative correlation between H3K27me3 enrichment at the CREM promoter and CREM mRNA levels in SLE CD4+ T cells ($r=-0.796$, $P<0.001$) [Figure 1: see original paper].

2.3 JMJD3, UTX, and EZH2 Levels at the CREM Promoter in SLE and Control CD4+ T Cells

ChIP combined with real-time quantitative PCR revealed significantly increased JMJD3 binding at the CREM promoter in SLE patients'CD4+ T cells compared with controls ($P<0.001$) [Figure 2A: see original paper]. In SLE CD4+ T cells, JMJD3 levels at this region negatively correlated with H3K27me3 ($r=-0.803$, $P<0.001$) [Figure 2B: see original paper] and positively correlated with CREM mRNA levels ($r=0.697$, $P<0.001$) [Figure 2C: see original paper]. However, no significant differences were observed in UTX ($P=0.172$) or EZH2 ($P=0.281$) binding at the CREM promoter between SLE patients and normal controls [Figure 2A: see original paper].

Discussion

Autoimmunity in SLE patients is driven by hyperactivated CD4+ T cells that stimulate B cells, resulting in excessive production of various autoantibodies. Epigenetic alterations at immune-related gene promoters in CD4+ T cells represent an important mechanism underlying this hyperactivation. However, most studies have focused on DNA methylation, with limited investigation of histone modifications in SLE CD4+ T cells.

H3K27me3 is known to suppress gene transcription by binding to Pc proteins in PRC1, thereby recruiting PRC1 to chromatin. PRC1 blocks binding of transcriptional activators and chromatin remodeling factors to DNA, impedes RNA polymerase II-mediated transcription, associates with histone deacetylases, and prevents positive activation marks such as H3K4 methylation. Consequently,

H3K27me3 has been a major focus in epigenetic research. To investigate whether H3K27me3 levels at gene promoters differ between SLE patients and normal controls, we screened H3K27me3 levels across various gene promoters in CD4+ T cells using ChIP microarray. We found reduced H3K27me3 at the CREM promoter in SLE patients, which aligns with elevated CREM expression in these cells.

While the role of CREM in SLE has been extensively characterized, the molecular mechanisms causing increased CREM in SLE T cells remain unclear. Our ChIP microarray results suggested that reduced H3K27me3 at the CREM promoter might lead to elevated CREM levels. We validated this finding using ChIP combined with real-time quantitative PCR, confirming significantly lower H3K27me3 at the CREM promoter in SLE CD4+ T cells. Moreover, we observed a negative correlation between H3K27me3 and CREM mRNA levels, indicating that reduced H3K27me3 contributes to CREM overexpression in SLE CD4+ T cells.

We next investigated why H3K27me3 levels are reduced at the CREM promoter in SLE CD4+ T cells. As mentioned, H3K27me3 levels are coordinately regulated by the demethylases JMJD3 and UTX and the methyltransferase EZH2. Using ChIP combined with real-time quantitative PCR, we examined these three H3K27 methylation-regulating enzymes at the CREM promoter and found significantly increased JMJD3 binding in SLE CD4+ T cells. JMJD3 levels negatively correlated with H3K27me3 and positively correlated with CREM mRNA levels. In contrast, no significant differences were observed in UTX or EZH2 binding at the CREM promoter between SLE patients and controls.

Collectively, our findings suggest that increased JMJD3 binding at the CREM promoter in SLE CD4+ T cells reduces local H3K27me3 levels, thereby promoting CREM overexpression. This epigenetic alteration may represent an important mechanism in SLE pathogenesis, providing new theoretical insights and potential therapeutic targets for SLE treatment.

Interestingly, while H3K27me3 blocks positive activation marks like H3K4 methylation, our group previously demonstrated that H3K4me3 levels at the CREM promoter are significantly elevated in SLE CD4+ T cells. The current finding of reduced H3K27me3 at the same locus raises the question of whether these two modifications are causally related or independent events, warranting further investigation.

References

[1] Moulton VR, Holcomb DR, Zajdel MC, et al. Estrogen upregulates cyclic AMP response element modulator alpha expression and downregulates interleukin-2 production by human T lymphocytes [J]. Mol Med, 2012, 18: 370-8.

[2] Tenbrock K, Kyttaris VC, Ahlmann M, et al. The cyclic AMP response

element modulator regulates transcription of the TCR zeta-chain[J]. J Immunol, 2005, 175(9): 5975-80.

[3] Hewagama A, Richardson B. The genetics and epigenetics of autoimmune diseases[J]. J Autoimmun, 2009, 33(1): 3-11.

[4] Pan Y, Sawalha AH. Epigenetic regulation and the pathogenesis of systemic lupus erythematosus[J]. Transl Res, 2009, 153(1): 4-10.

[5] Brooks WH, Le Dantec C, Pers JO, et al. Epigenetics and autoimmunity[J]. J Autoimmun, 2010, 34(3): J207-19.

[6] Zhang Q, Long H, Liao J, et al. Inhibited expression of hematopoietic progenitor kinase 1 associated with loss of jumonji domain containing 3 promoter binding contributes to autoimmunity in systemic lupus erythematosus[J]. J Autoimmun, 2011, 37(3): 180-7.

[7] Zhang P, Su Y, Lu Q. Epigenetics and psoriasis[J]. J Eur Acad Dermatol Venereol, 2012, 26(4): 399-403.

[8] Hong S, Cho YW, Yu LR, et al. Identification of JmjC domain-containing UTX and JMJD3 as histone H3 lysine 27 demethylases[J]. Proc Natl Acad Sci USA, 2007, 104(47): 18439-44.

[9] De Santa F, Totaro MG, Prosperini E, et al. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing[J]. Cell, 2007, 130(6): 1083-94.

[10] Banka S, Lederer D, Benoit V, et al. Novel KDM6A (UTX) mutations and a clinical and molecular review of the X-linked Kabuki syndrome (KS2)[J]. Clin Genet, 2015, 87(3): 252-8.

[11] Choi HJ, Park JH, Park M, et al. UTX inhibits EMT-induced breast CSC properties by epigenetic repression of EMT genes in cooperation with LSD1 and HDAC1[J]. EMBO Rep, 2015, 16(10): 1318-28.

[12] Fujii S, Ito K, Ito Y, et al. Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation[J]. J Biol Chem, 2008, 283(25): 17324-32.

[13] Hedrich CM, Rauen T, Kis-Toth K, et al. cAMP-responsive element modulator alpha (CREM) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE)[J]. J Biol Chem, 2012, 287(7): 4715-25.

[14] Hedrich CM, Crispin JC, Rauen T, et al. cAMP response element modulator alpha controls IL2 and IL17A expression during CD4 lineage commitment and subset distribution in lupus[J]. Proc Natl Acad Sci USA, 2012, 109(41): 16606-11.

[15] Rauen T, Hedrich CM, Juang YT, et al. cAMP-responsive element modulator (CREM) alpha protein induces interleukin 17A expression and mediates

epigenetic alterations at the interleukin-17A gene locus in patients with systemic lupus erythematosus[J]. J Biol Chem, 2011, 286(50): 43437-46.

[16] Ohl K, Wiener A, Schippers A, et al. Interleukin-2 treatment reverses effects of cAMP-responsive element modulator alpha-over-expressing T cells in autoimmune-prone mice[J]. Clin Exp Immunol, 2015, 181(1): 76-86.

[17] Gomez-Martin D, Diaz-Zamudio M, Crispin JC, et al. Interleukin 2 and systemic lupus erythematosus: beyond the transcriptional regulatory net abnormalities[J]. Autoimmun Rev, 2009, 9(1): 34-9.

[18] Koga T, Hedrich CM, Mizui M, et al. CaMK4-dependent activation of AKT/mTOR and CREM-alpha underlies autoimmunity-associated Th17 imbalance[J]. J Clin Invest, 2014, 124(5): 2234-45.

[19] Crispin JC, Tsokos GC. IL-17 in systemic lupus erythematosus[J]. J Biomed Biotechnol, 2010, 2010: 943254.

[20] Nalbandian A, Crispin JC, Tsokos GC. Interleukin-17 and systemic lupus erythematosus: current concepts[J]. Clin Exp Immunol, 2009, 157(2): 209-15.

[21] Tenbrock K, Juang YT, Leukert N, et al. The transcriptional repressor cAMP response element modulator alpha interacts with histone deacetylase 1 to repress promoter activity[J]. J Immunol, 2006, 177(9): 6159-64.

[22] Rauen T, Grammatikos AP, Hedrich CM, et al. cAMP-responsive element modulator alpha (CREM) contributes to decreased Notch-1 expression in T cells from patients with active systemic lupus erythematosus (SLE)[J]. J Biol Chem, 2012, 287(51): 42525-32.

[23] Verjans E, Ohl K, Yu Y, et al. Overexpression of CREM in T cells aggravates lipopolysaccharide-induced acute lung injury[J]. J Immunol, 2013, 191(3): 1316-23.

[24] Lippe R, Ohl K, Varga G, et al. CREM overexpression decreases IL-2 production, induces a TH17 phenotype and accelerates autoimmunity[J]. J Mol Cell Biol, 2012, 4(2): 121-3.

[25] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus[J]. Arthritis Rheum, 1997, 40(9): 1725.

[26] Zhao M, Sun Y, Gao F, et al. Epigenetics and SLE: RFX1 downregulation causes CD11a and CD70 overexpression by altering epigenetic modifications in lupus CD4+ T cells[J]. J Autoimmun, 2010, 35(1): 58-69.

[27] Lu Q, Wu A, Tesmer L, et al. Demethylation of CD40LG on the inactive X in T cells from women with lupus[J]. J Immunol, 2007, 179(9): 6352-8.

[28] Lund AH, van Lohuizen M. Polycomb complexes and silencing mechanisms[J]. Curr Opin Cell Biol, 2004, 16(3): 239-46.

[29] Kondo Y, Shen L, Cheng AS, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation[J]. Nat Genet, 2008, 40(6): 741-50.

[30] Zhang Q, Ding S, Zhang H, et al. Increased Set1 binding at the promoter induces aberrant epigenetic alterations and up-regulates cyclic adenosine 5' - monophosphate response element modulator alpha in systemic lupus erythematosus[J]. Clin Epigenetics, 2016, 8: 126.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.