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Research Article

Histone Lysine Demethylase 5A Promotes the Proliferation and Inhibits the Apoptosis of B Cells in SLE

Jie Fan[1], Kaimin Zhang[2], Farooq Riaz[2], Yiping Hu[3,4], Jinjun Liu[1], Qinwen Wang[3,4] and Fan Pan[2]*

[1]*Department of Cardiology, Handan Central Hospital, China*

[2]*Center for Cancer Research, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China*

[3]*Department of Rheumatology and Immunology, Peking University Shenzhen Hospital, Shenzhen, Guangdong, China*

[4]*Shenzhen Key Laboratory of Immunity and Inflammatory Diseases, Shenzhen, Guangdong, China*

***Corresponding Author:** Fan Pan, Center for Cancer Research, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China.

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Abstract

A classic autoimmune connective tissue disease, Systemic lupus erythematosus (SLE), causes injury to multiple organ systems. It has been demonstrated that tumor necrosis factor-induced protein 3 (TNFAIP3), typically represented as A20, is associated with the progression of SLE. However, it hasn't been revealed how A20 is involved in modulating the role of A20 in SLE. In the current study, we determined that A20 was reduced in B cells collected from SLE patients, while the responsiveness of B cells was considerably elevated in SLE patients. Further investigation indicated overexpressing A20 in B cells restricted their proliferation and initiated their apoptosis. Moreover, trimethylation of histone H3 Lysine 4 (H3K4me3) was decreased in the A20 promoter of SLE B cells. Lysine demethylase 5A (Kdm5a) was significantly increased in B cells from SLE patients and negatively correlated with A20 expression. Further, Kdm5a knockdown increased the H3K4me3 level and A20 expression. More importantly, Kdm5a promoted the proliferation and inhibited the apoptosis of B cells in SLE via downregulation of A20. In general, Kdm5a promoted the proliferation and inhibited the apoptosis of B cells in SLE via downregulation of A20 by decreasing H3K4me3 enrichment level in the A20 promoter, suggesting a novel mechanism underlying SLE progression, and providing a promising therapeutic target for SLE.

Keywords: Systemic Lupus Erythematosus; Tumor Necrosis Factor-Induced Protein 3; B Cells; Lysine Demethylase 5A

Introduction

Systemic lupus erythematosus (SLE) is characterized as one of the classic autoimmune connective tissue diseases which has detrimental effects on the injury of numerous organs, including muscles, joints, skin, and internal organs [1,2]. It has been elucidated that alongside being a multi-gene hereditary disease, SLE encompasses a high degree of clinical heterogeneity. Moreover, SLE is characterized by anomalous induction of T cells and response cell-mediated immunity, which deploys the immunopathological injury in various organs [3]. It was depicted that females, especially those in their childbearing age, exhibit a higher incidence of SLE than males [4]. Various factors, including genetic, environmental, and hormonal factors, are accountable for SLE [5]. Despite the extensive advances in medical and biological

sciences, the deep understanding of the pathological mechanism and effective therapies for SLE need further studies [6].

As an essential regulator of the immune response, B cells are pivotal in both acquired and natural immunity. B cells induce specific immune responses by acting as antigen-presenting cells (APCs) upon exposure to the abnormity of the environment. Meanwhile, B cells also exhibit themselves as the effector cells of humoral immunity by producing antigen-specific antibodies [7]. Thus, B cells are generally associated with the pathogenesis of SLE [8]. With the development of SLE, T cell dysfunction promotes uncontrolled B cell proliferation [9]. In contrast, over-hyperplasia of B cells enhances the autoantibodies production, which can bind the complement factors and corresponding autoantigens to form the immune complex [10,11]. Accumulation of these immune complexes in the vital organs can lead to immunopathological injuries in multiple organ systems [12]. Thus, altering the biological function of B cells is vital to medicate SLE.

Tumor necrosis factor-induced protein 3 (TNFAIP3), generally named A20, is recognized as a ubiquitin editing enzyme that acts as an essential player in modulating the pathogenesis of deregulatory immune diseases [13]. By employing the inhibition of A20, it has been well established that a decrease in A20 leads to the overactivation of B cells [14]. Remarkably, A20 was apparently reduced in the PBMCs (peripheral blood mononuclear cells) collected from the SLE patients [15]. In addition, A20 gene polymorphisms are testified to be firmly related to the vulnerability of SLE [16]. Considering these indications, we affirm that A20 plays critical role in the pathogenesis of SLE. However, in-depth studies describing the role of A20 in the progression of SLE are still limited.

Lysine demethylase 5A (Kdm5a) is a gene belonging to Jumonji family, AT-rich interactive domain 1 histone demethylase protein family. Kdm5a is responsible to control numerous cellular processes through demethylase-dependent regulation of gene expressions [17,18]. Additionally, Kdm5a is well described to employ a definite impact on the trimethylation of histone H3 Lysine 4 (H3K4me3). Briefly, Kdm5a catalyzes the H3K4me3 demethylation of the targeted gene promoter, thereby reducing the targeted gene expressions [19].

In the current study, we aimed to explore the role of A20 in the progression of SLE as along with the detailed molecular mechanism underlying its functions. These data revealed that A20 was repressed in the B cells collected from the SLE individuals, and the overexpression of A20 controlled the growth of B cells and enhanced the apoptosis in the B cells. More prominently, Kdm5a overexpression elevated the growth of B cell and diminished the apoptosis of B cells in SLE individuals by subsiding the A20 via reducing H3K4me3 amelioration level in the A20 promoter, signifying an un-described mechanism underlying SLE pathogenesis.

Materials and Methods

Cell isolation and culture

In this study, blood samples from 23 SLE and 15 healthy individuals were collected. All the patients met at least four of the classification standards of SLE as anticipated by the American College of Rheumatology. All the samples were attained after informed consent was obtained from each individual patient. The ethics committee of Handan Central Hospital approved the study.

PBMCs were isolated by Lymphoprep (Axis-Shield) using the density gradient centrifugation of heparinized blood. Later, PBMCs were proceeded for B cell separation by utilizing the CD19 Dynabeads from Dynal (Oslo, Norway). The purity of the collected B cells was over 90%, as specified by flow cytometric analysis. The collected B cells were cultured in the cRPMI-1640 growth media supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine, and activated by CpG ODN 2006 (1 μM; InvivoGen, San Diego, CA, USA) for 48 hours. B cells were discretely cultured from three SLE patients, which were all labeled as SLE B cells. While normal B cells cultured were collected from three healthy individuals.

Cell transfection

The full-length coding sequence of human kdm5a or A20 was cloned into the widely available mammalian overexpression pcDNA3.1 plasmid vector to create pcDNA-kdm5a and pcDNA-A20 plasmids, respectively. For experimental control, empty pcDNA3.1 plasmid was utilized. Meanwhile, si-A20, si-kdm5a, and si-negative control (NC) were purchased from GenePharma (Suzhou, China). Lipofectamine 3000 was utilized for the transfection of these siRNAs or plasmids into normal B cells or SLE B cells (Invitrogen, San Diego, CA, USA) following the manufacturer's description.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

RNA isolation was done by utilizing the TRIzol reagent (Invitrogen), following the indications of the reagent. The isolated RNA was checked for integrity and quantity and utilized to investigate the expression of A20, Kdm5a, CD40, CD80, and CD86 by using the One Step PrimeScript™ RT-PCR kit (Takara, Dalian, China). The following primers used in our study: A20 (F: 5'-CACGCTCAAGGAAACAGACA-3', R: 5'-CATGGGTGTGTCTGTGGAAG-3'), CD40 (F: 5'-CCTCGCCATGGTTCGTCTGCC-3', R: 5'-AGCCAGGAAGATCGTCGGG-3'), CD86 (F:5'-GGACTAGCACAGACACACGGA-3', R: 5'-CTTCAGAGGAGCAGCACCAGA-3'), CD80 (F: 5'-GCAGGGAACATCACCATCCA-3', R: 5'-TCACGTGGATAACACCTGAACA-3'), kdm5a (F: 5'--GATGACAGCATGGAAGAGAAAC-3', R: 5'-GCCAGTTTATTCAGCTCCTTTG-3') and β-actin (F: 5'-CGCGAGAAGATGACCCAGAT-3', R: 5'-GCACTGTGTTGGCGTACAGG-3'). Gene expression was analyzed by using the $2^{-\Delta\Delta Ct}$ method, while β-actin was used as housekeeping control.

Western blot

Protein was extracted using RIPA buffer (KeyGEN BioTECH, Nanjing, China), and quantification was done by using the BCA Protein Assay kit (Solarbio, Beijing, China), by following the instruction of the company's manual. The isolated proteins were subjected to electrophoresis using sodium dodecylsulphate polyacrylamide (SDS-PAGE) gel, followed by allocation onto polyvinylidene fluoride (PVDF) membranes. Then membranes were blocked with 5% skim milk, and probed with the primary antibodies against A20, IκBβ, IκBε, kdm5a and β-actin for 14 h at 4°C, followed by immunoblot with horseradish peroxidase (HRP)-conjugated second antibody. Antibodies used here were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The blots were established with an ECL chemiluminescence kit from Beyotime (Shanghai, China) and examined employing Image J software.

Chromatin immunoprecipitation (ChIP)-qPCR

ChIP assay kit (Millipore, Billerica, MA, USA) was programmed to reveal the relationship between A20 promoter and trimethylation of histone H3 Lysine 9 (H3K9me3), histone 3 acetylation (H3Ac), and trimethylation of histone H3 Lysine 4 (H3K4me3). B cells were separated as described early, and fixed with 37% formaldehyde. After 10 min of incubation, the cells were dealt with glycine for quenching formaldehyde. Then cells proceeded for centrifugation and incubation with SDS-Lysis Buffer. The cell lysates were sonicated to achieve the DNA fragments of 200-1000 bp sizes. Sheared chromatin was centrifuged to the pellet and treated with the antibodies against H3Ac, H3K4me3 and H3K9me3 for 14 h at 4°C. Subsequently, reaction mixture was precipitated with protein-A agarose beads. Beads were centrifuged, and Precipitated DNA was eluted and recovered using phenol/chloroform. DNA quantification was achieved by real-time PCR by using the A20 promoter region primers, as previously described [20], and assayed for H3Ac, H3K4me3 and H3K9me3 levels.

Determination of nuclear factor-kappaB (NF-κB) activity

The NF-κB activity was calculated by using the luciferase assays through a Dual-Luciferase reporter assay system (Promega, Madison, WI, USA). In brief, B cells were plated in 6-well plates, and transfected with Renilla luciferase vector, pRL-TK, and NF-κB reporter plasmid using Lipofectamine 3000 reagent. After incubating in media containing Lipofectamine 3000 reagent, we replaced the media containing Lipofectamine 3000 reagent, and then transfected cells with pcDNA-A20, pcDNA-kdm5a, si-A20, si-kdm5a or the matched controls. After 48 h of incubation, the cells were used to calculate NF-κB activity using the Dual Luciferase reporter assay system.

5-ethynyl-2'-deoxyuridine (EdU) assay

B cells were harvested after transfection, and incubated with EdU solution (Solarbio) for 2 h. Later, B cells were fixed for 30 min by using 4% paraformaldehyde, followed by incubating with glycine for 5 min with vigorous shaking. Afterwards, cells were washed with PBS and incubated for 10 min with 0.5% TritonX-100. This was followed by incubation with Apollo dye in the shady place, and washed with 0.5% TritonX-100 twice. Finally, cells were incubated with DAPI for 30 min, washed with PBS, and analyzed using a fluorescence microscope.

Flow cytometry

Cells were isolated and centrifugated for 5 min at 1000 *g* after transfection. The supernatant was wasted, and the pellet was taken

and softly dissolved in PBS solution. Subsequently, the cells were pelleted and dissolved in Annexin V-FITC 5solution (Beyotime). After incubation, cells were stained with propidium iodide for 15 min at 25°C in the dark to analyze the apoptosis rate. Additionally, to evaluate the levels of CD40, CD86, and CD80 in B cells, flow cytometry was carried out using anti-human CD40, anti-human CD86, or anti-human CD80 antibody (Abcam, Cambridge, MA, USA). A flow cytometer (BD Biosciences, San Jose, CA, USA) was applied to analyze the CD40, CD80 and CD86 levels.

Statistical analysis

Our data were scrutinized through SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA) by employing student's *t* test for two groups or one-way ANOVA followed by Tukey post-hoc test for various groups. All the data are presented as the mean ± standard error of the mean, and a significant difference was established as $P < 0.05$.

Results

A20 is significantly declined in B cells from SLE patients

As assessed by qRT-PCR, the level of A20 was evidently reduced in B cells at both transcriptional and translational level B cells isolated from SLE patients as compared to B cells collected from healthy individuals (Figure 1A and B). In addition, we also established that there was an increase in the activity of NF-κB in B cells from SLE patients compared to B cells from healthy individuals (Figure 1C).

Figure 1: A20 is significantly decreased in B cells from SLE patients. (A and B) qRT-PCR and western blot assay were separately undertaken to examine the expression of A20 mRNA and protein in B cells from SLE patients and healthy volunteers. (C) The NF-κB activity was detected using a PiccaGene Luciferase Assay Kit. ***P < 0.001.

B cell reactivity was suggestively elevated in SLE individuals

As represented in Figure 2A, the mRNA expression of CD40 and CD86 were outstandingly amplified in B cells from SLE individuals when compared with the healthy individuals, while no noteworthy alteration was observed in CD80 expression in B cells from SLE patients and healthy individuals. Similar results were attained after the flow cytometry assay (Figure 2B).

Figure 2: B cell responsiveness was significantly elevated in SLE patients. qRT-PCR assay (A) and flow cytometry (B) were conducted to determine the expression of CD40, CD80 and CD86 mRNA in B cells from SLE patients and healthy volunteers. *P < 0.05 and ***P < 0.001.

A20 reduces proliferation and activates apoptosis of B cells

To understand the role of A20 in the pathogenesis of SLE, we performed gain- and loss-of-function analysis by transfecting A20 overexpression plasmid or si-A20 into SLE B cells or Normal B cells. The results of qRT-PCR and western blot analysis indicated that the A20 expression was downregulated in normal B cells transfected with si-A20 compared to cells stimulated with si-NC, but was noticeably augmented in B cells from SLE patients transfected with pcDNA-A20 than that pcDNA-NC transfected B cells (Figure 3A and 3B). Furthermore, an increase in A20 led to an elevated NF-κB activity in Normal B cells; however, knockdown of A20 caused the decrease of NF-κB activity in B cells from SLE patients (Figure 3C). Consistent with this, forced downregulation of A20 decreased IκBβ and IκBε expression in Normal B cells, while A20 overexpression enhanced the level of IκBβ and IκBε in SLE B cells (Figure 3D). Simultaneously, loss of A20 increased the growth of Normal B cells (Figure 3E), as evaluated by EdU assay. Besides, overexpression of A20 hindered the growth of SLE B cells (Figure 3F). Meanwhile, we also found that a decrease in A20 restrained the Normal B cells apoptosis, but forced upregulation of A20 increased the apoptosis of SLE B cells, as determined by flow cytometry analysis (Figure 3G and 3H).

Figure 3: A20 inhibits proliferation and induces apoptosis of B cells. We transfected pcDNA-A20 into SLE B cells and introduced si-A20 into Normal B cells. (A) qRT-PCR and (B) western blot assays were performed to examine the expression of A20 in SLE B cells and Normal B cells. (C) The activity of NF-κB was detected in SLE B cells and Normal B cells. (D) Western blot was conducted for the detection of IκBβ and IκBε in SLE B cells and Normal B cells. (E and F) EdU assay was undertaken to assess the proliferation of SLE B cells and Normal B cells. (G and H) Flow cytometry was applied to measure the apoptosis of SLE B cells and Normal B cells. *P < 0.05, **P < 0.01 and ***P < 0.001.

H3K4me3 was reduced in A20 promoter of SLE B cells

To assess the effect of histone amendment on the A20 expression in SLE B cells, we achieved ChIp-qPCR assay, and examined the H3Ac, H3K4me3 and H3K9me3 expressions in association with A20 promoter in B cells from SLE and healthy individuals. As expected, the level of H3K4me3 in the promoter region of the A20 gene considerably decreased in B cells from SLE individuals compared to B cells from healthy individuals. Whereas, no considerable difference was observed in H3Ac and H3K9me3 levels in B cells from SLE patients and healthy individuals (Figure 4).

Figure 4: H3K4me3 was decreased in A20 promoter of SLE B cells. ChIp-qPCR assay was carried out to measure the levels of H3Ac, H3K4me3 and H3K9me3 at A20 genomic loci in B cells from SLE patients and healthy volunteers. *P < 0.05.

Kdm5a was significantly increased in B cells from SLE patients and negatively correlated with A20 expression

To verify if the decrease of H3K4me3 is due to the decrease of H3K4 methyltransferase or the elevated H3K4 demethylation, we resoluted the level of 14 H3K4 methyltransferases (Mll1, MII2, Mll3, Mll4, Mll5, Setd1a, Setd1b, Setd7 and Ashl1) and H3K4 demethylations (Kdm1a, Kdm5a, Kdm5b, Kdm5c and Kdm5d) in SLE B cells and Normal B cells by employing qRT-PCR assay (data not shown). qRT-PCR data indicated that the mRNA level of Kdm5a was clearly augmented in SLE B cells than Normal B cells (Figure 5A). Meanwhile, a negative correlation was noticed between the A20 expression and Kdm5a expression in SLE B cells and Normal B cells (Figure 5B and 5C).

Kdm5a knockdown enhanced the H3K4me3 level and A20 expression

To further evaluate the effect of Kdm5a on the levels of H3K4me3 and A20, we used pcDNA-Kdm5a and si-Kdm5 to transfect Normal B cells and SLE B cells, respectively. As determined by qRT-PCR

Figure 5: Kdm5a was significantly increased in B cells from SLE patients and negatively correlated with A20 expression. (A) qRT-PCR assay was done to measure the levels of Kdm5a in SLE B cells and Normal B cells. (B) The association between A20 expression and Kdm5a expression was determined in SLE B cells. (C) The association between A20 expression and Kdm5a expression was determined in Normal B cells. ***P < 0.001.

and western blot, the level of Kdm5a was significantly upregulated in Normal B cells transfected with pcDNA-Kdm5a compared with the controls. However, level of Kdm5a was noticeably decreased in SLE B cells transfected with si-Kdm5 (Figure 6A). ChIP-qPCR data indicated that overexpression of Kdm5a downregulated the level of H3K4me3 in Normal B cells, while silencing of Kdm5a enhanced the expression of H3K4me3 in SLE B cells (Figure 6B). Additionally, enhanced Kdm5a led to the reduced A20 expression in Normal B cells, while si-Kdm5a led to an elevated A20 expression in SLE B cells (Figure 6C). Besides, increased Kdm5a upregulated the NF-κB activity in Normal B cells, while reduced Kdm5a expression declined the NF-κB activity in SLE B cells (Figure 6D). Consistent with this, we revealed that the IκBβ and IκBε levels were decreased in Normal B cells after transfection with pcDNA-Kdm5a, but was enhanced in SLE B cells which were transfected with si-Kdm5a, as examined by western blot (Figure 6E).

Kdm5a promotes proliferation and inhibits apoptosis in B cells via downregulation of A20

To further verify the role of Kdm5a in promoting the proliferation and inhibiting the apoptosis of B cells via targeting A20, we used pcDNA-Kdm5a or si-Kdm5a and NCs pcDNA-A20 or si-A20 to transfect Normal B cells and SLE B cells. EdU assay exposed that upregulation of Kdm5a endorsed the growth of Normal B cells, which was restricted by the forced increase in A20 expression

Figure 6: Kdm5a knockdown increased the H3K4me3 level and A20 expression. we transfected pcDNA-Kdm5a into Normal B cells to overexpress Kdm5a in Normal B cells and transfected si-Kdm5 into SLE B cells to knock Kdm5a in SLE B cells. qRT-PCR assay was done to measure the levels of Kdm5a (A) and A20 mRNA (C) in SLE B cells and Normal B cells. (B) ChIp-qPCR assay was carried out to measure the level of H3K4me3 in SLE B cells and Normal B cells. (D) The activity of NF-κB was measured in SLE B cells and Normal B cells. (E) Western blot was conducted for the detection of IκBβ and IκBε in SLE B cells and Normal B cells. *P < 0.05, **P < 0.01 and ***P < 0.001.

along with the overexpression of Kdm5a (Figure 7A). Conversely, inhibiting Kdm5a suppressed the proliferation of SLE B cells, and this suppression was alleviated by inhibiting A20 in the presence of si-Kdm5a (Figure 7B). Likewise, increased Kdm5a decreased the apoptosis of SLE B cells, and this phenomenon was overturned after pcDNA-A20 co-transfection with pcDNA-Kdm5a (Figure 7C). Silencing of Kdm5a endorsed the apoptosis of SLE B cells, which was revoked by A20 silencing in the cells co-transfected with si-Kdm5a (Figure 7D).

Discussion and Conclusion

A20 has been well established to serve as the negative mediator of NF-κB in response to numerous stimuli. A20 acts as ubiquitin-editing enzyme which has capability to undo K63-linked ubiquitin

Figure 7: Kdm5a promotes proliferation and inhibits apoptosis in B cells via downregulation of A20. We transfected Normal B cells and SLE B cells with pcDNA-Kdm5a or si-Kdm5a and pcDNA-A20 or si-A20. (A and B) EdU assay was conducted to assess the proliferation of Normal B cells and SLE B cells. (C and D) The apoptosis of Normal B cells and SLE B cells was determined by flow cytometry. *P < 0.05 and **P < 0.01.

chains from receptor interacting protein-1 or TNF receptor associated factor 6 involved in the NF-κB signaling pathway; thus, terminates NF-κB signaling [21]. For instance, A20 decrease and UBCH7 increase synergistically persuaded the stimulation of NF-κB, and then led to the secretion of inflammatory cytokines, thereby increasing the overall SLE risk [22]. Decreasing A20 targets the tumor necrosis factor-α (TNF-α)-induced receptor-mediated inhibition of NF-κB signaling and then contributes to modulating autoimmunity [23]. Moreover, anomalous lessening of A20 tempted by TNF-α triggered the reduction of A20 in SLE monocytes, which arbitrated the NF-κB activation, resulting in the extended inflammatory response in SLE [24]. However, it is not understood how A20 plays its role in mediating the functions of B cells from SLE patients. Herein, the downregulation of A20 was disclosed in B cells collected from SLE patients, complementary to the activation of NF-κB. Furthermore, enhanced A20 diminished the activation of NF-κB, and hindered and enhanced the growth and apoptosis of B cells in SLE patients, respectively. This was consistent with the initial studies indicating the role ofA20 in impairing the B cell survival and endangered against autoimmunity [25].

Even though several studies have associated A20 with the B cell functions in SLE patients, it is still not investigated why A20 is

decreased in the B cells from SLE patients. Epigenetic modification is known as a heritable gene transcriptional regulation resulting without the changes in the sequence of genomic DNA, including histone modification, DNA methylation and chromatin remodeling, which exerts critical impact in diverse physio and pathological processes [26,27]. Recently, histone modification has emerged as an attractive subject in the epigenetic-related research [28]. Abnormal histone modifications have been associated with the development of numerous human diseases [29]. Among these histone modifications, lysine methylation is described as most illustrative histone modification until recently [30]. Precisely, methylation of H3K4 linked to transcriptional activation exerts important role in the modulation of mitosis-related genes, such as Hox and TBX families. Meanwhile, it has been elucidated that the decrease of A20 in CD4+ T cells isolated from SLE patients might diminish the methylation of histone H3K4 in the A20 promoter [31]. However, little is known about whether the decrease of A20 is due to histone methylation modification. In our study, we selected three common patterns of histone methylation (H3Ac, H3K4me3 and H3K9me3) to investigate the contribution of histone methylation in the reduction of A20 in B cells from SLE patients. Our results exposed that the expression of H3K4me3 in the A20 promoter region considerably decreased in B cells from SLE patients, demonstrating the association of decreased H3K4me3 in the A20 promoter with the decreased A20 in B cells from SLE individuals.

Additional investigation was carried out to elevate the decreased H3K4me3 in the A20 promoter. To verify if the lessening of H3K4me3 is due to the diminished H3K4 methyltransferase or the increased of H3K4 demethylation, we examined the level of H3K4 methyltransferases and H3K4 demethylations, and revealed that Kdm5a were enhanced in B cells from SLE individuals. We also revealed that the expression of Kdm5a was negatively associated with A20 expression in SLE B cells and Normal B cells. In addition, we exposed that Kdm5a knockdown considerably elevated the H3K4me3 levels and A20 expression, while overexpression of Kdm5a increased the proliferation and reduced the apoptosis of B cells in SLE by downregulating A20, thereby suggesting a novel mechanism underlying SLE pathogenesis.

Overall, we found that A20 is downregulated in B cells from SLE patients, and the forced expression of A20 restrains the proliferation and induces the apoptosis of B cells in SLE patients. Mechanistically, the decrease of A20 in linked with the B cells from SLE patients is due to the inhibition of H3K4me3 in the A20 promoter. More precisely, Kdm5a was enhanced in B cells from SLE patients and endorsed the growth and reduces apoptosis of B cells in SLE by downregulating A20, signifying a novel mechanism underlying SLE pathogenesis and providing a promising therapeutic target for SLE.

Ethics Approval and Consent to Participate

The present study was approved by Handan Central Hospital, and written informed consent was obtained from all participants.

Consent for Publication

All patients in this study provided their consent for publication.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its additional files.

Declarations of Interest

There is no conflict interest existed.

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