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Ultraviolet B enhances DNA hypomethylation of CD4+ T cells in systemic lupus erythematosus via inhibiting DNMT1 catalytic activity

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ABSTRACT

Background: CD4+ T cells DNA hypomethylation is involved in the pathogenesis of systemic lupus erythematosus (SLE). Recent studies showed that ultraviolet B (UVB, 290–320 nm) might induce the exacerbation of SLE by decreasing the DNA methylation level. However, the role of DNA methyltransferase 1 (DNMT1) in the UVB-induced CD4+ T cells DNA hypomethylation remains unclear.
Objective: To elucidate the role of DNMT1 in lupus CD4+ T cells global DNA hypomethylation enhanced by UVB.
Methods: 35 SLE patients and 15 healthy controls were enrolled in the study. CD4+ T cells from SLE patients and healthy controls exposed to different dosages of UVB were analyzed. The global DNA methylation measurement, real-time PCR, Western blotting and DNMT1 catalytic activity detection were employed.
Results: The level of global DNA methylation and DNMT1 mRNA expression in CD4+ T cells from SLE patients were significantly lower than those from the control group. DNA methylation was decreased after UVB exposure in a dosage-dependent manner in SLE patients, but not in the control group. DNMT1 mRNA and protein expression level were not affected by UVB exposure in both SLE patients and healthy controls. DNMT1 catalytic activity was significantly decreased in CD4+ T cells from SLE patients after UVB exposure in a dosage-dependent manner. DNMT1 catalytic activity was lower and more sensitive to UVB exposure in CD4+ T cells from active SLE patients that from stable ones.
Conclusion: UVB enhanced DNA hypomethylation of CD4+ T cells in SLE via inhibiting DNMT1 catalytic activity in a dosage-dependent manner.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease characterized by uncontrolled lymphocyte autoreactivity that triggers inflammation and tissue damage in many parts of the body. The molecular mechanisms initiating the autoimmune response are poorly understood, although both genetic and epigenetic factors are implicated [1]. A large body of evidence implicates DNA hypomethylation is involved in the pathogenesis of SLE. For example, inhibiting DNA methylation in normal CD4+ T cells induces autoreactivity, and these autoreactive cells promote autoantibody production [2].

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) including DNMT1, DNMT3A and DNMT3B [3,4]. The foremost contributor in the mammalian methylation scheme is DNMT1, a maintenance methyltransferase that faithfully copies the pre-existing methyl marks onto hemimethylated daughter strands during DNA replication to maintain the established methylation patterns across successive cell divisions [5]. The ever-changing cellular physiology and the significant part that DNA methylation plays in genome regulation necessitate rigid management of this enzyme. Both expression and catalytic activity of DNMT1 are essential for the maintenance of global DNA methylation pattern [6]. A host of DNMT1 inhibitors, such as procainamide and hydralazine have shown the ability to lead to DNA hypomethylation and induce SLE [7,8].

Ultraviolet (UV) light is one of the most potential inducers of SLE. Up to 73% of patients with SLE report photosensitivity [9]. Most cutaneous lupus lesions can be triggered by sunlight exposure. Sunlight exposure, especially ultraviolet B light (UVB, 290–320 nm), can even induce systemic disease activity. A link between UV exposure, defective post-replication repair and altered methylation has been reported: UV exposure was shown to lead to specific demethylation events during subsequent rounds of replication [10]. As a consequence of aberrant repair or repair methylation, UV exposure has also been used to activate the transcription of a quiescent metallothionine gene, which based on 5-azacytidine-reactivation experiments, is thought to be under

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methylation control [11]. However, the underlying mechanism that UV induces DNA hypomethylation is still unknown.

In this study, we set out to investigate the influence of UVB on the expression and catalytic activity of DNMT1 and to elucidate the mechanism that DNA hypomethylation induced by UVB. We report here, for the first time, that UVB inhibited DNMT1 catalytic activity, but not the DNMT1 expression in CD4+ T cells from SLE patients, and subsequently enhanced DNA hypomethylation.

2. Materials and methods

2.1. Subjects

Thirty-five SLE patients (30 female and 5 male, mean age: 33.40 ± 1.55) were recruited from outpatient department of Shanghai First People's Hospital. All patients were newly diagnosed according to the 1997 ACR revised criteria for classification of SLE [12] and did not receive any treatment. Disease activity was assessed by using the SLE disease activity index (SLEDAI) and active disease was defined as an SLEDAI score ≥5. Age- and sex-matched healthy controls (12 female and 3 male, mean age: 30.42 ± 1.79) were recruited from medical staff at the Shanghai First People's Hospital. Relevant clinical and laboratory information regarding the study subjects were shown in Table 1. This study was approved by the human ethics committee of the Shanghai Jiaotong University, and written informed consent was obtained from each subject.

2.2. Isolation and culture of CD4+ T cells

20 ml of peripheral venous blood samples were collected from each subject in tubes containing ethylene diamine tetraacetic acid (EDTA) anticoagulant. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density-gradient centrifugation (Eppendorf, Germany). CD4+ T cells were isolated by positive selection using magnetic beads (Miltenyi Biotec, Germany) and the purity was evaluated by flow cytometry (purity was generally higher than 95%; Becton Dickinson, USA). The cells were then cultured in human T cell culture medium (Lonza, USA).

2.3. UVB exposure

UVB treatment of cells was performed by using Waldman UV109B lights with TL-12 lamps (Waldman Lighting Ltd., Germany) emitting primarily in the 290–320 nm range with an emission peak at 310 nm. Cells were irradiated in phosphate buffered saline (PBS) with $Mg2^{+}/Ca2^{+}$ at a distance of 40 cm. UVB dosage used in this study were 20, 40 or 80 mJ/cm^2. Sham-irradiated cells were handled similarly, but shielded with an aluminum foil against UVB exposure. PBS was removed after irradiation, and then human T cell culture medium was added and the cells were cultured for 24 h. Dosage of UVB was chosen based on the World Health Organization guidelines for sun exposure and on the standard erythemal dose (SED), a cumulative measure of erythemal or sunburning solar UV irradiation [13,14].

Table 1
Clinical and laboratory characteristics of the subjects.

Characteristic	SLE (n = 35)	Control (n = 15)
Male/female (n)	5/30	3/12
Age (years)	33.40 ± 1.55	30.42 ± 1.79
SLEDAI score	7.00 ± 0.85	NA
Active/stable (n)[a]	21/14	NA
Anti-dsDNA (IU/ml)	303.80 ± 24.27	NA
C3 (g/l)	0.45 ± 0.02	NA
C4 (g/l)	0.09 ± 0.01	NA
Red cell count ($\times 10^{12}$/l)	3.22 ± 0.08	NA
Lymphocyte count ($\times 10^{9}$/l)	2.00 ± 0.06	NA
Platelet count ($\times 10^{9}$/l)	194.73 ± 6.57	NA
Lupus nephritis [P/N (n)]	11/24	NA
Facial rashes [P/N (n)]	13/22	NA

SLEDAI = Systemic Lupus Erythematosus Disease Activity Index. Value are presented as mean ± SEM, except where indicated otherwise. P/N: positive/negative.
[a] Active disease was defined as an SLEDAI score ≥5.

2.4. Global DNA methylation measurement

Global DNA methylation was evaluated by staining the cells with specific monoclonal antibody against 5-methylcytidine (Acris Antibodies, Germany) using the previously published protocols [15,16]. CD4+ T cells were washed with PBS supplemented with 0.1% Tween 20 and 1% bovine serum albumin (PBST-BSA), fixed with 0.25% paraformaldehyde at 37 °C for 10 min and 88% methanol at −20 °C for at least 30 min. After two washes with PBST-BSA, the cells were treated with 2 M HCl at 37 °C for 30 min and then neutralized with 0.1 M sodium borate (pH8.5). The cells were blocked with 10% goat serum in PBST-BSA for 20 min at 37 °C, incubated with anti-5-methylcytidine antibody (1 μg/ml) for 45 min at 37 °C followed by staining with goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Santa Cruz Biotechnology, USA). Finally, the cells were washed with PBS three times and resuspended in PBS for analysis by flow cytometry (BD, USA). Data were acquired and analyzed using CellQuest Software (BD, USA) and Flowjo Software.

2.5. Reverse transcription-PCR and qRT-PCR analysis

Total RNA was extracted using RNeasy Mini kit (Qiagen, USA). Reverse transcription was performed using PrimeScript RT-PCR kit (Takara Bio, Japan). qRT-PCR was performed on the Mx3000p real-time system (Stratagene, USA) using SYBR Premix Ex Taq (Takara Bio, Japan). Amplification was started at 95 °C for 30 s as the first step, followed by 40 successive cycles of PCR: at 95 °C for 5 s, at 60 °C for 20 s. The primers from Takara Bio were as follows:

DNMT1 forward: 5′-GCACCTCATTTGCCGAATACA-3′;
reverse: 5′-TCTCCTGCATCAGCCCAAATA-3′;
β-actin forward: 5′-ACCCAGATCATGTTTGAGACC-3′;
reverse: 5′-AGGGCATACCCCTCGTAGA-3′.

2.6. Western blotting analysis

CD4+ T cells were incubated with Complete Lysis-M (Roche Applied Science, USA). Lysate protein concentration was measured using BCA Protein Assay kit (Pierce, USA). Equal amounts of protein (20 μg) were dissolved in NuPage LDS Sample Buffer (Invitrogen, USA) and 10% NuPage Sample Reducing Agent (Invitrogen, USA). Lysates were boiled at 70 °C for 10 min and loaded and run on 4–12% NuPage Bis-Tris Gels (Invitrogen, USA) at 200 V for 40 min. The proteins were transferred onto polyvinylidene fluoride membranes (Invitrogen, USA) and blocked in 2% BSA in 0.1% Tween-20 (Sigma–Aldrich, USA) and Tris-buffered saline. Membranes were probed with anti-DNMT1 rabbit IgG antibody (ab19905, Abcam, USA) or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) rabbit IgG antibody (FL-335, Santa Cruz Biotechnology, USA) overnight at 4 °C. The secondary antibody used was anti-rabbit horseradish peroxidase-conjugated IgG antibody. Protein bands were detected using the Western Breeze kit (Invitrogen, USA). Densitometric analysis of protein band was performed using ImageJ software (NIH).

2.7. Nuclear extraction and DNMT1 catalytic activity detection

Nuclear extracts were prepared by using EpiQuik™ Nuclear Extraction Kit I (Epigentek Group Inc., USA). DNMT1 activity was