Biomedicine & Pharmacotherapy 99 (2018) 791–797

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

miR-125b is downregulated in systemic lupus erythematosus patients and inhibits autophagy by targeting UVRAG

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ARTICLE INFO

Keywords:
Ultraviolet B
miR-125b
Systemic lupus erythematosus
UV radiation resistance associated gene
Autophagy

ABSTRACT

Systemic lupus erythematosus (SLE) is a severe autoimmune disease and the pathogenesis remains incompletely understood. This study aimed to investigate the role of miR-125b in the pathogenesis of SLE and explore the underlying mechanism. Compared to healthy controls, the expression of miR-125b decreased in peripheral blood mononuclear cells (PBMCs) of SLE patients. In addition, PBMCs exposed to ultraviolet B had lower miR-125b level compared to those unexposed to radiation. We identified UV radiation resistance associated gene (UVRAG) as a target of miR-125b. Jurkat cells treated with miR-125b-5p agomir showed reduced levels of ATG7, Beclin-1 and LC3 II and decreased autophagy. In contrast, Jurkat cells treated with miR-125b-5p antagomir showed increased levels of ATG7, Beclin-1 and LC3 II and increased autophagy. Furthermore, Jurkat cells transfected with UVRAG expression vector showed higher expression of ATG7, Beclin-1 and LC3 II and increased autophagy. Conversely, cells transfected with UVRAG siRNA had lower expression of ATG7, Beclin-1 and LC3 II and decreased autophagy. Taken together, our data demonstrate that Ultraviolet B radiation can downregulate miR-125b-5p and increase UVRAG expression and autophagy activity in PBMCs of SLE patients. These findings help explain how ultraviolet B exacerbates SLE and suggest that UVRAG is a potential therapeutic target for SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is the most common autoimmune disease. Although the pathogenesis of SLE is still unclear, peripheral blood mononuclear cells (PBMCs) have been implicated in SLE [1]. In addition, ultraviolet exposure especially ultraviolet B (UVB) is considered a significant immunomodulatory factor that can exacerbate pre-existing lupus [2]. Interestingly, UVB radiation has been shown to upregulate miR-125b level in psoriasis patients [3]. Notably, miR-125b was reported to be downregulated in PBMCs in SLE patients [4]. These results suggest that miR-125b may inhibit the development of SLE, but the underlying mechanism remains elusive.

Autophagy is crucial to cell homeostasis and malfunction of autophagy pathway has been shown to be related to many autoimmune diseases, including SLE [5]. Autophagy related genes are significantly upregulated in PBMCs of SLE patients [6]. UV radiation resistance associated gene (UVRAG) was first isolated from xeroderma pigmentosum as a cDNA partially complementing UV sensitivity, but recent evidences suggest its crucial role in the regulation of autophagy [7]. UVRAG has been reported as a target gene of miR-125a, and miR-125a mediated downregulation of Uvrag led to the inhibition of autophagy [8]. Considering that miR-125a and miR-125b are homolog, we speculated that miR-125b could target UVRAG.

In this study we aimed to investigate the role of miR-125b in the pathogenesis of SLE and explore the underlying mechanism. First, we compared miR-125b expression levels in PBMCs from SLE patients and healthy controls subjected to UVB radiation. Next, we verified that UVRAG was a target gene of miR-125b-5p. Moreover, we found that miR-125b-5p inhibitor or UVRAG overexpression promoted autophagy.

2. Methods

2.1. Subjects

Twelve SLE patients (mean age: 33.08 ± 9.46, male/female: 1/11, mean SLEDAI: 4.75 ± 2.52, range dosage of predenisolone: 5–15 mg/d) were recruited who visited Department of Dermatology at the second affiliated hospital of Kunming Medical University from 2015/12/01 to 2016/07/31. All SLE patients met the classification criteria of American Rheumatism Association revised in 1997 and 2009. Since hydroxychloroquine and chloroquine may depress autophagy, patients taking these drugs in the last three months were excluded. Twelve healthy

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https://doi.org/10.1016/j.biopha.2018.01.119
Received 18 December 2017; Received in revised form 12 January 2018; Accepted 24 January 2018

controls (mean age: 32 ± 7.42, male/female: 1/11) who underwent routine physical examinations were recruited as NC group. The study protocols were approved by the Ethics Committee of Kunming Medical University, and written informed consent was obtained from all subjects.

2.2. Cell culture and UVB radiation

15 ml of blood sample was obtained from each subject and collected in sterile tubes containing EDTA. PBMCs were separated by using Human Lymphocyte Separation Medium, and cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) (Gibco-BRL) and antibiotics (Gibco, Grand Island, NY, USA) at 37 ℃ in a humid atmosphere with 5% CO2. 293T cells and Jurkat cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) and RPMI-1640 medium, respectively, containing 10% FBS and antibiotics (Gibco, Grand Island, NY, USA) at 37 ℃ in a humidified atmosphere with 5% CO2 [9]. For UVB exposure, the cells were radiated by UVB (220 V/50 HZ, 5 cm, 50 mj/cm^2).

2.3. Luciferase assay

Wild type or mutant 3′UTR of UVRAG was cloned into pmiR-RB-Reporter vector (Ribobio, Guangzhou, China). 293T cells were co-transfected with reporter and miR-125b-5p mimic or mimic negative control (Ribobio, Guangzhou, China) by Lipofectamine 2000 (Invitrogen). After 48 h, the cells were harvested to examine luciferase activity by a dual luciferase assay system (Promega, Madison, WI, USA).

Jurkat cells were transfected with miR-125b-5p agomir/antagomir or miR-Control agomir/antagomir by Lipofectamine 2000 (Invitrogen). Jurkat cells were electroporated at 180 V 15 ms (ECM 830 Electroporation System, Holliston MA, USA) with pIRES2-EGFP-UVRAG or siRNA UVRAG (Ribobio, Guangzhou, China) according to the manufacturer's instructions. After transfection for 48 h, the cells were harvested to perform western blot, RT-qPCR and fluorescent LC3 puncta tests.

2.4. Real-time PCR

Total RNA and miRNA were isolated from cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. After synthesizing cDNAs with All-in-One First-Strand cDNA Synthesis Kit (GeneCopoeia, MD, USA), the expression levels of miR-125b-5p, UVRAG, ATG7 and Beclin-1 were analyzed using All-in-One qPCR Mix (GeneCopoeia, MD, USA) and run with Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Data were analyzed by $2-\Delta\Delta$ CT method and presented relative to the expression of GAPDH for UVRAG, ATG7 and Beclin-1 and in relation to the expression of small nuclear U6 RNA for miR-125b-5p.

2.5. Western blot analysis

For protein isolation from PBMCs and Jurkat cells, RIPA buffer (Sigma-Aldrich, St. Louis, MO) supplemented with proteinase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) was used. The protein concentration was determined using the BCA Protein Assay Kit (Pierce). Equal amounts of cell lysates were loaded on a 10% (16% for LC3) sodium dodecyl sulfate-polyacrylamide gel for electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Millipore, IL, USA). The membranes were blocked for 1 h at room temperature using Tris-buffered saline with 0.05% Tween 20 (TBST) and 5% skimmed milk, and then the following primary antibodies were applied overnight at 4 ℃: anti-UVRAG (Cell Signaling Technology), anti-ATG7 (Abcam), anti-Beclin-1 (Santa Cruz), anti-LC3B (Sigma-Aldrich, St. Louis, MO) and anti-β-actin (Santa Cruz). After washing three times with TBST, the membranes were incubated with secondary antibody at room temperature for 1 h and washed again with TBST [10]. Images of target proteins were detected by Chemiluminescent HRP Substrate Kit (Millipore, IL, USA).

2.6. Lentiviral infection and confocal fluorescence microscopy

Jurkat cells were labeled with LentiBrite™ RFP-LC3 Lentiviral Biosensor Packaged Lentiviral Particles (Millipore, IL, USA) according to the manufacturer's instructions to establish RFP-LC3 Jurkat cell line. Cells were fixed in 4% paraformaldehyde and the nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St. Louis, MO) for 10 min before imaging. A FV10-ASW laser scanning confocal fluorescence microscope (Olympus Corp, MPE FV1000) was used to acquire image.

2.7. Statistical analysis

All the experiments were repeated three times. Statistical analyses were performed using SPSS 22.0 for Windows software package. All data were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA with LSD test as post-hoc test. $p < .05$ was regarded as statistically significant.

3. Results

3.1. UVB decreased miR-125b level and upregulated autophagy proteins in PBMCs from SLE patients

First, we performed real-time PCR to detectmiR-125b expression level in PBMCs from SLE patients. The results showed that miR-125b level was significantly lower in PBMCs from SLE patients than in PBMCs from healthy controls. In addition, miR-125b levels significantly decreased in PBMCs from both SLE patients and healthy controls after exposure to UVB (Fig. 1a).

Next we examined the effects of UVB exposure on the expression of autophagy proteins in PBMCs. Western blot analysis showed that protein levels of UVRAG, Beclin-1, ATG7 and LC3-II showed significant increase in PBMCs from SLE patients after exposure to UVB, but only showed slight increase in PBMCs from health controls (Fig. 1b). Furthermore, RT-PCR confirmed that mRNA levels of UVRAG, Beclin-1 and ATG7 significantly increased in PBMCs from SLE patients after exposure to UVB, but only slightly increased in PBMCs from health controls (Fig. 1c–e). These data reveal the link between decreased miR-125b level and increased level of UVRAG in PBMCs from SLE patients after UVB exposure.

3.2. UVRAG is a target gene of miR-125b

To characterize the negative correlation between miR-125b and UVRAG expression levels, we performed bioinformatic analysis using TargetScan and PicTar algorithm and predicted that UVRAG is a target of miR-125b. Next, we subcloned wild type or mutant 3′UTR of UVRAG into a pmiR-RB-Report luciferase reporter (Fig. 2a). Luciferase reporter assay in 293T cells demonstrated that miR-125b-5p mimic significantly decreased luciferase activity driven by UVRAG 3′UTR wild type construct, while the mutant construct showed no response to miR-125b-5p mimic (Fig. 2b).

To examine whether UVRAG is a target of miR-125b in PBMCs, we transfected miR-125b-5p agomir or miR-Control agomir into Jurkat cells. qPCR and Western blot analysis showed that mRNA and protein levels of UVRAG increased after UVB exposure but decreased by miR-125b-5p agomir both in the absence of UVB exposure and in the presence of UVB exposure. (Fig. 2c,d). Furthermore, we transfected miR-125b-5p antagomir or control antagomir into Jurkat cells and found that mRNA and protein levels of UVRAG increased by miR-125b-5p antagomir (Fig. 2e,f). Taken together, these data suggest that UVRAG is