BBA - Gene Regulatory Mechanisms 1861 (2018) 773–782

Contents lists available at ScienceDirect

BBA - Gene Regulatory Mechanisms

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

LPS promotes HBO1 stability via USP25 to modulate inflammatory gene transcription in THP-1 cells

Chen Long[a,b], Yandong Lai[b], Jin Li[b], Jiangsheng Huang[a,*], Chunbin Zou[b,*]

[a] Department of Minimally Invasive Surgery, the Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China
[b] Acute Lung Injury Center of Excellence, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

ARTICLE INFO

Keywords:
HBO1
USP25
Ubiquitination
Inflammation
Gene transcription

ABSTRACT

The histone acetyltransferase HBO1 (Histone acetyltransferase binding to origin recognition complex 1, Myst2/Kat7) participates in a range of life processes including DNA replication and tumorigenesis. Recent studies revealed that HBO1 is involved in gene transcriptional activation. However, the molecular behavior of HBO1 in inflammation is yet to be studied. Here we report that endotoxin lipopolysaccharide (LPS) elevates HBO1 protein level via up-regulating UPS25 (ubiquitin specific peptidase 25) and alters inflammatory gene transcription in THP-1 monocytes and in human primary macrophages. LPS protects HBO1 from ubiquitin proteasomal degradation without significantly altering its transcription. By immunoprecipitation, we identified that HBO1 associates with a deubiquitinating enzyme USP25 in THP-1 cells. LPS increases protein level of USP25 resulting in accumulation of HBO1 by suppression of HBO1 ubiquitination. Stabilized-HBO1 modulates inflammatory gene transcription in THP-1 cells. These findings indicate that USP25 promotes stability of HBO1 in bacterial infection thereby enhances HBO1-mediated inflammatory gene transcription.

1. Introduction

HBO1 (histone acetyltransferase binding to origin recognition complex 1, Myst2/Kat7) is a member of the histone acetyltransferase family. As a major histone acetylation enzyme, HBO1 is involved in a range of life processes. In the late M to early G1 phase of cell cycle, HBO1 incorporates with Cdt1, docking onto the chromatin to acetylate histone H3 at K14 and H4 at K12 [1,2]. Histone H3/H4 acetylation triggers DNA replication licensing thereby initiating DNA replication [3,4]. It has been reported that HBO1 overexpresses in many cancer cells and is related to aberrant progression of cell cycle and unlimited proliferation [5–9]. HBO1 plays a role in DNA repair after ultraviolet radiation [10]. The acetyltransferase activity of HBO1 is required for ubiquitin proteasomal degradation of the estrogen receptor, indicating HBO1 acetylates non-histone proteins as well [9,11]. In HBO1-knockout mice, peripheral CD4+ and CD8+ T cells are substantially reduced which indicates HBO1 is critical in T cell development [12]. Recent studies indicate that HBO1 may modulate gene transcription. Knockout of HBO1 reduced > 90% of the acetylation in H3K14, which is required for H3K14-dependent transcriptional activation of a panel of important genes for embryo development [13]. HBO1 directly interacts with transcription factor SIX1 and another histone acetyltransferase NCOA-3 to promote expression of corresponding glycolytic genes [14]. However, the role of HBO1 in inflammatory gene transcription in immune cells is not fully studied.

Post-translational modifications such as phosphorylation and ubiquitination have been reported in HBO1. A protein microarray analysis revealed that HBO1 is a substrate of the cyclin E/CDK2 complex. The cyclin E/CDK2 complex phosphorylates HBO1 at T88 which promotes the enrichment of cancer stem-like cells in breast cancer [15]. Phosphorylation of HBO1 at S57 by Polo-like kinase augments multi-drug resistance in pancreatic cancer cells [7]. Our previous work reported that HBO1 is an unstable protein with a half-life of approximate 3 h in lung epithelial cells. HBO1 is poly-ubiquitinated and regulated at the

Abbreviation: HBO1, histone acetyltransferase binding to origin recognition complex 1; UPS25, ubiquitin specific peptidase 25; LPS, lipopolysaccharide; Cdt1, Chromatin Licensing And DNA Replication Factor 1; HDAC11, histone deacetylase 11; MCM, Minichromosome Maintenance Complex; SIX1, Sine Oculis Homeobox Homolog1; NCOA-3, Nuclear Receptor Coactivator 3; CDK2, Cyclin Dependent Kinase 2; SCF, Skp, Cullin, F-box containing complex; DUB, Deubiquitinating enzyme; H3K4me3, histone H3 lysine 4 trimethylation; H3K27me3, histone H3 lysine 27 trimethylation; acH3K14, histone H3 lysine 14 acetylation; DsiRNA, Dicer-Substrate Short Interfering RNAs; CHX, Cycloheximide; TLR4, Toll Like Receptor 4; MyD88, Myeloid Differentiation Primary Response 88; TRIF, TIR-domain-containing adapter-inducing interferon-β

* Corresponding authors.
E-mail addresses: HJS13907313501@csu.edu.cn (J. Huang), zouc@upmc.edu (C. Zou).

https://doi.org/10.1016/j.bbagrm.2018.08.001
Received 27 April 2018; Received in revised form 30 July 2018; Accepted 2 August 2018
Available online 04 August 2018
1874-9399/

protein level by SCF-Fbxw15-mediated ubiquitin proteasomal degradation [16]. The molecular mechanisms of HBO1 regulation in distinct pathophysiological settings has yet to be understood.

Protein poly-ubiquitination is a reversible post-translational modification, a group of deubiquitinating enzymes remove the poly-ubiquitin chain. According to their distinct structures, deubiquitinating enzymes can be categorized into six sub-families: the ubiquitin-specific proteases (USPs), the ovarian tumor proteases (OTUs), the ubiquitin Carboxyl-terminal hydrolases (UCHs), the Josephin family, the motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDYs), and the Zn-dependent JAB1/MPN/MOV34 metalloprotease DUBs (JAMMs) [17]. USP25, a member of ubiquitin-specific proteases sub-family, hydrolyzes both K48 and K63 linkages of polyubiquitin moieties where link to the substrate protein [18]. USP25 stabilizes tankyrases to enhance the Wnt/β-catenin signaling contributing to cancer development [19]. Aberrant expression of USP25 has been noted in breast cancer and non-small cell lung cancer patients as well [20,21]. Interestingly, USP25 is a type 1 interferon responding gene; both viral infection and LPS treatment up-regulate USP25 expression [22–24]. Nevertheless, the signal transduction of USP25 under viral and bacterial infection requires further study.

Epigenetic mechanisms have exclusively and controversially emerged in every aspect of immunity including immune cell activation, lineage development and regulatory cell function [25,26]. Among them, monocyte activation and proliferation are critical steps corresponding to bacterial infection [27]. Mounting evidence shows that epigenetics modulates host innate immunity against exogenous pathogens through multi-channel mechanisms involving in DNA methylation, histone post-translational modification and the activity of non-coding RNAs [28,29]. For example, in mycobacterium tuberculosis infection, hyper-methylation located in promoter regions of IL-17 family members has been observed in THP-1 monocytes [30]. Additionally, Histone H3K4me3 and H3K27me3 negatively regulate differentiation of monocytes in dendritic cells [31]. The molecular behavior of HBO1 in macrophage or monocyte in responding to bacterial infection has yet to be elucidated.

2. Materials and methods

2.1. Cell line and reagents

Human monocyte THP-1 cells were cultured in RPMI1640 medium (500 mL, ATCC 30-2001) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) Non-Essential Amino Acid (NEAA), 1% (v/v) sodium pyruvate, and 100 μg/mL streptomycin and 100 Units/mL penicillin. Human primary macrophages and their culture medium were from Celprogen (San Pedro, CA) and maintained in a 37 °C incubator in the presence of 5% CO_2.

The antibody against HBO1 (1:2000, cat#: ab70183) and recombinant human USP25 protein (Cat#: ab188736) were purchased from Abcam. AcH3K14 antibody (1:1000, cat#: A-4023-050) were purchased from EpiGentek. Lamin A/C antibody was purchased from Cell signaling (1:1000, cat#:4C11). Antibodies purchased from Santa Cruz Biotechnology are listed in Table 1. β-actin antibody (1:10000, cat#: A5441, lot#: 116M4801V), LPS (cat#: L4391, lot#: 067M4036V) derived from *Escherichia. coli* (serum type: O111:B4), Actinomycin D (cat#: A9415, lot#: 026M4034V), and Leupeptin (cat#: L2884) were purchased from Sigma Aldrich. Protein A/G Pierce™ Protein A/G Agarose (cat#: 20422, lot#: SH254756) and Completed protease inhibitor cocktail (cat#: 88266, lot#: QE20062910) were purchased from Pierce. MG132 (cat#: F1101, lot#: F11042823) was purchased from UBPBio. Cycloheximide (cat#: ALX-380-269-G001) and ubiquitin aldehyde (cat#: BML-UW8450-0050, lot#: 07051723) were purchased from Enzo life Science. SYBR™ Select Master Mix (cat#: 4472920) was purchased from Applied Biosystem.

2.2. Plasmid and DsiRNA transfection

DsiRNA or *plasmids* were transfected into THP-1 cells using electroporation executed with a nuclear transfection apparatus (Amaxa Biosystem, Gaithersburgh, MD, USA). Dicer-Substrate Short Interfering RNAs (DsiRNA) against *HBO1* or *USP25*, including *USP25-DsiRNA-1, USP25-DsiRNA-2, USP25-DsiRNA-3, HBO1-DsiRNA* and *Scrambled-DsiRNA* (cat#: 51-01-14-03), were purchased from Integrated DNA Technology (IDT). *pCMV6-XL5/HBO1 plasmid* (catalog No: SC115703) and *pCMV6-XL5/USP25 plasmid* (catalog No: SC115248) were purchased from Origene. Briefly, one million cells in 100 μL of transfection buffer (20 mM HEPES in PBS buffer) were mixed with 10 pM of DsiRNA or 1 μg of plasmid. RPMI 1640 medium (2 mL) was added to the six-well plates or flasks for each cuvette after the electroporation. Preset program U-001 was used for THP-1 cells. Transfected cells were cultured 72 h for further treatment or analysis.

2.3. Immunoblotting and co-immunoprecipitation

Immunoblotting and co-immunoprecipitation were conducted as previously described [32]. Briefly, cell lysates were prepared with lysis buffer (1X PBS, 1: 500 proteinase inhibitor cocktail, 0.3% of SDS). Whole cell lysates were pre-cleared and applied to SDS-PAGE. The proteins were transferred to membranes, blocked with 5% (w/v) non-fat milk in Tris-buffered saline, and probed with primary antibodies as indicated. An enhanced chemiluminescence (ECL) was used to develop the image and the images were acquired with a Kodak in vivo professional 4000 system. For immunoprecipitation, 1 mg of cell lysates (in PBS with 0.5% Tween 20, and protease inhibitors) were incubated with specific primary antibodies for overnight at 4 °C. The mixture was added to 40 μL of protein A/G-agaroses for an additional 2 h at 4 °C. The precipitated complex was washed three times with 0.5% Tween 20 in PBS and analyzed by immunoblotting as described above. In HBO1-Ub co-immunoprecipitation experiment, cells were pretreated with MG132 for 30 min, and ubiquitin aldehyde (5 μM) was added to the cell lysis buffer to inhibit DUB activity [33].

2.4. Subcellular protein fractionation

THP-1 cells were collected by centrifugation at 1500 rpm for 5 min and washed with cold PBS buffer. The pelleted cells were resuspended in 1 mL of cytoplasmic membrane lysis buffer (10 mM Hepes, pH 8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.1% Igepal CA-630, and protease inhibitor cocktails) and incubated on ice for 10 min. The cell suspensions were subjected to centrifuge at 2000 rpm for 5 min, and the supernatant was collected as the cytoplasmic proteins and condensed by Amicon Ultra-4 Centrifugal filters (Merck Millipore Ltd). The pellets were lysed with 100 μL of cell lysis buffer (1X PBS, 1: 500 proteinase inhibitor cocktail, 0.3% of SDS). After centrifuging at 13,000 rpm for 10 min, the supernatants were collected as the nuclear proteins. The subcellular proteins were subjected to immunoblotting analysis.

2.5. In vitro de-ubiquitination assay

Endogenous HBO1 protein was obtained by HBO1 immunoprecipitation as described above. Briefly, THP-1 cells were treated with MG132 for 30 min prior to harvest and cell lysates were prepared with lysis buffer (1X PBS, 1: 500 proteinase inhibitor cocktail, 0.3% of SDS). The cell lysates (contains 1 mg total protein) were incubated with HBO1 antibody (2 μg) for 2 h followed by Protein A/G agarose beads (30 μL) incubation (1 h). The protein A/G agarose beads were precipitated by centrifugation and washed once with de-ubiquitination buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, pH 8.00, 10 mM DTT). The beads in a 25 μL of de-ubiquitination buffer were incubated for 4 h at 37 °C in the presence or absence of active recombinant human USP25 protein (3 μg, as recommended by the