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Repression of a chromatin modifier aggravates lipopolysaccharide-induced acute lung injury in mouse

Jia-kuan Chen [a,1], Wen-chen Wang [a,1], Li Zang [b], Jie Zhao [c], Wei Li [c,*], Tao Jiang [a,**]

[a] Department of Thoracic Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China
[b] Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China
[c] Department of Histology and Embryology, Fourth Military Medical University, Xi'an 710032, China

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ABSTRACT

Local inflammatory responses and alveolar epithelial cells (AECs) apoptosis are both important for the development of the acute lung injury (ALI), a clinically important complication causing high morbidity and mortality, but little is known about the molecular mechanisms underlying the pathogenesis. Herein, we showed for the first time that expression of Metastasis-associated protein 1 (MTA1), a master transcriptional regulator with the ability to regulate divergent cellular pathways by modifying the acetylation status of crucial target genes, was up-regulated in the alveolar cells of the *Escherichia coli* lipopolysaccharide (LPS)-induced murine ALI model. Inhibition of MTA1 expression by *in vivo* siRNA treatment exacerbated the pathology of LPS-induced ALI, by selectively promoting the expression of NF-κB-regulated inflammatory cytokines. Moreover, ablation of MTA1 expression promoted the LPS-induced apoptosis in AEC II cells, leaving AEC I cells unaffected. These data collectively underscore an alveolar facet of this important chromatin modifier, which may represent as a novel regulator and a new therapeutic target for the treatment of ALI.

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1. Introduction

The acute lung injury (ALI), along with its severe phase acute respiratory distress syndrome (ARDS), a clinically important complication and a significant cause of high morbidity and mortality in critically ill patients. Common causes of ALI include sepsis, trauma, aspiration, multiple blood transfusion, acute pancreatitis, inhalation injury, and certain types of drug toxicity [1]. Histologically, ALI/ARDS is characterized by a severe acute inflammatory response, a massive apoptosis in alveolar epithelial cells (AECs), a profound increase in alveoli-capillary permeability, and subsequent formation of fibrosis [2]. Although the physiological and cellular responses to ALI have been well documented, the molecular mechanisms through which these responses are directed remain largely unknown [3].

Metastasis-associated protein 1 (MTA1), the prototype of the MTA family, along with other MTA members, regulates cellular pathways by associating and modifying the acetylation status of the target gene chromatin. MTA1 functions in conjunction with distinct histone deacetylase (HDAC) to mediate transcriptional regulation and it serves as both a transcriptional repressor and a transcriptional activator [4]. Although MTA1 has been linked intimately with human cancer, its role in inflammatory responses remains unrecognized until the recent identification of the critical homeostatic role of MTA1, both as a target and as a component of the nuclear factor-kappaB (NF-κB) circuitry, in the regulation of *Escherichia coli* lipopolysaccharide (LPS)-elicited inflammatory responses in macrophages [5]. Nevertheless, the role of this chromatin modifier in other inflammation-related pathologies remains largely unknown.

MTA1 expression is ubiquitous and in lung, it is mainly expressed in AECs [6], but the role of this important chromatin modifier in alveolar cells remains poorly addressed. It is well known that NF-κB plays an essential role in the pathogenesis of lung diseases and it is required for maximal transcription of numerous cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). All these inflammatory cytokines are important in the development of ALI [7]. From

* Corresponding author. Department of Histology and Embryology, Fourth Military Medical University, No. 169 Changle West Road, Xi'an 710032, China.
** Corresponding author. Department of Thoracic Surgery, Tangdu Hospital, Fourth Military Medical University, No. 1 Xinsi Road, Xi'an 710038, China.
E-mail addresses: liweipepeyato@163.com (W. Li), jiangtaochest@163.com (T. Jiang).
[1] These authors contributed equally to this work.

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a therapeutic standpoint, inhibition of NF-κB signaling could attenuate LPS-induced inflammatory responses, thus alleviating the pathogenesis of ALI [8]. Considering the close relationship between MTA1 function and recruitment of histone deacetylation complexes in the modulation of NF-κB cascade, we hypothesized that MTA1 may be potentially involved in ALI. An expression and mechanistic study comprising multiple analyses was therefore designed to elucidate the potential links between this chromatin modifier and the complicated nature of ALI. Our systematic analysis will pave the way for a better understanding of the role of MTA1 in alveolar biology.

2. Materials and methods

2.1. Mouse ALI model

All animal procedures were approved in advance by the local ethical committee. Adult female C57BL/6 mice were obtained from the Animal Research Center of our university. After 7 days of acclimatization, mice were challenged with intratracheal instillation of 800 μg of lipopolysaccharide (LPS, *E. coli* 055:B5; Sigma–Aldrich, Beijing, China) dissolved in 50 μl of normal saline. Naïve mice (without LPS challenge) were injected with saline only [9]. Mice were sacrificed 18 h following LPS instillation (n = 6 for each group) by CO_2 inhalation. For histological studies, lung tissues were fixed in 4% paraformaldehyde for 20 h, embedded in paraffin and processed into 5-μm-thick sections. Sections were then stained with hematoxylin and eosin. To calculate the lung injury score, two separate pathologists evaluated each slide in a blinded manner, following a previously described protocol [10].

2.2. Western blotting

Western blotting was carried out as described previously [11,12]. Briefly, protein samples were prepared in ice-cold RIPA buffer (Tris–HCl 50 mM, NaCl 150 mM, Triton X-100 1% vol/vol, sodium deoxycholate 1% wt/vol, and SDS 0.1% wt/vol pH 7.5) supplemented with complete proteinase-inhibitor cocktail tablets (Roche Diagnostic, Mannheim, Germany). 25 μg of protein sample were separated by SDS/PAGE and transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA). Membranes were then incubated with different primary antibodies including goat anti-MTA1 and rabbit anti-TUBULIN (Santa Cruz Biotechnology, Paso Robles, CA, USA). Immunostained bands were finally detected by using an ECL kit (Amersham Biosciences, Buckinghamshire, UK). Densitometric analysis of immunoblots was performed using Image J software (National Institutes of Health).

2.3. Immunohistochemistry

The avidin–biotin–peroxidase (ABC) method was employed in the immunohistochemical assay according to our previous work [13].

2.4. In vivo siRNA treatment

The specificity of the siRNA we used here has been confirmed in our previous work [14]. 40 μg siRNA was diluted with 50 μl 10% glucose solution, and sterile water was added to 100 μl and mixed gently. 6.4 μl *in vivo* transfection reagents (SignaGen Laboratories, Rockville, MD, USA) was diluted with 50 μl 10% glucose solution, and sterile water was added to 100 μl and mixed gently. The *in vivo* transfection reagents and the siRNA dilution were mixed quickly and gently, followed by the incubation at room temperature for 15 min siRNA was injected into mice through the tail vein 1 d before LPS challenge [15].

2.5. RT-qPCR

Total RNA was extracted from lung tissues using RNeasy Mini Kit (QIAGEN Inc., Valencia, CA, USA). For RT-PCR, first-strand cDNA was synthesized with Superscript III (Rnase H-Reverse Transcriptase; Invitrogen) and PCR was set up according to Promega's protocol. The primers used for detection *MTA1*, *IL-1β*, *IL-6*, *TNF-α*, *IL-10*, *ICAM-1*, *Ttf-1* and *Gapdh* were chosen according to previous reports [5,16,17]. Amplification of *Gapdh* was served as the internal control. PCR products were quantified by SYBR green intercalation using the MiniOpticon™ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). *Gapdh* was used to obtain the △△Ct values for the calculation of fold increases.

2.6. NF-κB activity

Nuclear extraction from lung tissues was carried out using a commercial kit from Signosis (Zhongzhi Biotech, Wuhan, China). Signosis' NF-κB filter assay was performed to evaluate the activity of NF-κB pathway as instructed by the manufacturer. The bound NF-κB probe was finally measured with luminescence using GloMax™ 20/20 Luminometer (Promega, Beijing, China).

2.7. In situ end labeling of fragmented DNA (TUNEL)

TUNEL assay was done using In Situ Cell Death Detection Kit (Roche Applied Science, Mannheim, Germany) following instructions of the manufacturer [18].

2.8. Statistical analysis

All graphed data represent three independent experiments performed in triplicate. Data are presented as mean ± S.E.M. with probability determined by *Student's t* test, with $P < 0.05$ being considered as statistically significant.

3. Results

3.1. Induction of MTA1 expression in mouse ALI model

Immunoblotting was firstly employed to evaluate the influence of ALI on MTA1 expression level in adult mouse lung. 18 h after LPS challenge, mice were sacrificed and lung tissues were harvested. MTA1 expression was significantly increased in the ALI group (Fig. 1A and B). The upregulation of MTA1 expression in lung mainly occurred in the AECs, as revealed by immunohistochemistry (Fig. 1C).

3.2. Effects of in vivo inhibition of MTA1 expression on ALI pathology

To investigate the role of endogenous MTA1 in the pathogenesis of ALI, we knocked down the expression of *Mta1* in adult lung by using a specific siRNA. *Mta1* siRNA and Ctrl siRNA were injected into mice through the tail vein 1 d before LPS challenge, according to a previously validated protocol [15]. *Mta1*/MTA1 depletion was confirmed by RT-qPCR (Fig. 2B) and western blotting (Fig. 2C). About 40% decrease of *Mta1*/MTA1 was detected at the end of 2 d after siRNA treatment. Unexpectedly, in the presence of siRNA treatment, even ALI could not elicit the induction of MTA1 expression. At 18 h following LPS insult, mice treated with *Mta1* siRNA (n = 6) had a significantly higher lung injury score than animals treated with the Ctrl siRNA (n = 6; $P < 0.05$). In the absence

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