EBioMedicine 10 (2016) 195–203

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Destabilization of Lysophosphatidic Acid Receptor 1 Reduces Cytokine Release and Protects Against Lung Injury

Jing Zhao [a,b,*], Jianxin Wei [a,b], Su Dong [a,b,c], Rachel K. Bowser [a,b], Lina Zhang [d], Anastasia M. Jacko [a,b], Yutong Zhao [a,b,**]

[a] Department of Medicine, University of Pittsburgh, School of Medicine, Acute Lung Injury Center of Excellence, Vascular Medical Institute, United States
[b] Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA, United States
[c] Department of Anesthesia, First Hospital of Jilin University, Changchun, China
[d] Department of Critical Care Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 2 July 2016
Accepted 15 July 2016
Available online 18 July 2016

Keywords:
GPCR
Ubiquitination
Deubiquitination
TLR4/CD14
Lung injury

ABSTRACT

Lysophosphatidic acid receptor 1 (LPA1) is a druggable target for treating pulmonary inflammatory diseases. However, the molecular regulation of LPA1 stability, a factor that critically impacts its biological activity, remains largely unknown. Here we identify two enzymes that regulate the balance of LPA1 ubiquitination and deubiquitination. Ubiquitin E3 ligase Nedd4L targets LPA1 for its site specific ubiquitination and degradation in the lysosome. Nedd4L negatively regulates LPA-LPA1-mediated cytokine release. The stability of LPA1 is up-regulated by ubiquitin-specific protease 11 (USP11), which deubiquitinates LPA1 and enhances LPA1-mediated pro-inflammatory effects. LPA1 is associated with USP11 in quiescent cells, while LPA treatment triggers LPA1 dis-association with USP11 and in turn binding to Nedd4L. Knockdown or inhibition of USP11 reduces LPA1 stability, levels of LPA1, and LPA1-CD14 interaction complex; thereby diminishing both LPA- and LPS-induced inflammatory responses and lung injury in preclinical murine models. Thus, our findings identify an ubiquitin E3 ligase and a deubiquitinating enzyme responsible for regulation of LPA1 stability and biological activities. This study provides potential targets for the development of anti-inflammatory molecules to lessen lung injury.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

In the United States, approximately 200,000 people suffer annually from acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS), with a high mortality rate ranging from 30 to 40% (Rubenfeld et al., 2005). Acute respiratory infection commonly elicits a cytokine storm, leading to epithelial and endothelial barrier disruption, edema, and often respiratory failure. Lipopolysaccharide (LPS), a bacterial endotoxin, induces a strong cytokine storm through its receptor complex composed of toll-like receptor 4 (TLR4) and CD14 (Lu et al., 2008; Ojaniemi et al., 2003; Park and Lee, 2013). In addition to binding to TLR4, CD14 associates with other cell surface proteins, such as surfactant protein C (Augusto et al., 2003), to modulate LPS-induced inflammatory responses. Our recent study revealed that CD14 interacts with the lysophosphatidic acid receptor 1 (LPA1), which belongs to G-protein-coupled receptors (GPCRs) (Zhao et al., 2011).

LPA1 is highly expressed in lung tissue and contributes to the pathogenesis of lung injury through binding to LPA (Zhao and Natarajan, 2013; Lin et al., 2010), a naturally occurring bioactive lipid, and its interaction with CD14 (Zhao et al., 2011). We have shown that LPA1-modulated signals are involved in release of chemotactic factor interleukin 8 (IL-8) (Saatian et al., 2006; Cummings et al., 2004; Zhao et al., 2005), neutrophil influx to the lungs (He et al., 2009), and airway epithelial hyperplasia (Funke et al., 2012). The protein level of LPA1 is increased in lung inflammatory disorders, including asthma (Georas et al., 2007), pulmonary fibrosis (Tager et al., 2008), and ALI (Zhao et al., 2011). LPS- or bleomycin-induced lung injury is diminished in LPA1-deficient mice (Zhao et al., 2011; Tager et al., 2008); therefore, LPA1 is a potential target for pharmaceutical treatment of lung injury. However, the molecular regulation of LPA1 has not been well studied.

The mono-ubiquitination-lysosome system degrades the majority of cell surface receptors including GPCRs (Marchese and Trejo, 2013; Marchese et al., 2003; Shenoy et al., 2008; Henry et al., 2012; Xiao and Shenoy, 2011). Three enzyme complexes (E1, E2, and E3) are involved in protein ubiquitination. E3 ubiquitin ligases facilitate the covalent attachment of ubiquitin to specific lysine residue(s) within target proteins. The E3 ubiquitin ligase for LPA1 has not been reported. Nedd4L, a member of HECT class of E3 ubiquitin ligase has been known to

* Corresponding author.
** Correspondence to: Y. Zhao, Department of Medicine, University of Pittsburgh, 3459 Fifth Avenue, NW 628 MUH, Pittsburgh, PA, 15213, United States.
E-mail addresses: zhaoj@upmc.edu (J. Zhao), zhaoy3@upmc.edu (Y. Zhao).

http://dx.doi.org/10.1016/j.ebiom.2016.07.020
2352-3964/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

catalyze ubiquitination of cell surface and intracellular proteins, such as epithelial sodium channel (ENaC) (Kamynina et al., 2001), Smad2, and Smad3 (Gao et al., 2009). Here we demonstrate that the ubiquitin E3 ligase Nedd4L mediates LPA1 ubiquitination and lysosomal degradation, therefore limiting LPA1-modulated signaling.

Protein ubiquitination is reversible; removal of ubiquitin chains from target proteins is mediated by deubiquitinating enzymes. We have shown that ubiquitin-specific protease (USP) 14 modulates I-κB levels (Mialki et al., 2013). Little is known about the role of USPs in the regulation of GPCR stability. USP11, a ubiquitous protein in various human cells, has been shown to enhance TGFβ receptor (ALK5) stability (Al-Salihi et al., 2012) and regulate DNA repair (Wiltshire et al., 2010). The current study reveals that LPA1 is a substrate for USP11, and inhibition of USP11 mitigates lung injury through reduction of LPA1 levels and LPA1-CD14 pathway.

2. Materials and Methods

2.1. Cells and Reagents

Murine lung epithelial (MLE12) cells (from ATCC) were cultured with HITES medium containing 10% fetal bovine serum (FBS) and antibiotics at 37 °C in 5% CO_2. Primary culture of human bronchial cells (HBEpCs) (from Lonza, Baltimore, MD) was conducted using medium supplemented with growth factors provided by Lonza. V5 antibody, mammalian expressional plasmid pcDNA3.1-His-V5-topo, and *Escherichia coli* Top10 competent cells were from Life technologies (Grand Island, NY). P-p38 MAPK, p38 MAPK, p-IκB, Nedd4L, HA tag, and ubiquitin antibodies were from Cell Signaling (Danvers, MA). LPA1 and LPA2 antibodies were from LifeSpan BioScience, Inc. (Seattle, WA). Cycloheximide (CHX, 3-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide), leupeptin (Acetyl-Leu-Leu-Arg-al), lipopolysaccharide (LPS), β-actin and myc tag antibodies were from Sigma (St. Louis, MO). MG-132 (Z-L-Leu-D-Leu-L-Leu-al) and bafilomycin A1 (C35H58O9) were from EMD Chemicals (Philadelphia, PA). Immunobilized protein A&G beads, control IgG, p-Erk1&2, Erk1&2, and USP11 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). All materials in highest grades used in the experiments are commercially available.

2.2. Plasmid and shRNA Transfection

Human *LPA1*, human *NEDD4L*, or human *USP11* cDNA, and mutants were inserted into pCDNA3.1-V5-His-Topo vector, pCDNA3.1-HA vector, or pCDNA3.1-myc vector. All the primers were designed using Primer3 or QuickChange Primer Design Tool (Agilent Technologies Inc.) software. Over-expression of plasmids in MLE12 cells was performed using the Lonza nucleofector system. Over-expression of plasmids in HBEpCs was performed using FuGENE HD reagent (Promega, Madison, WI).

2.3. Preparation of Protein Extracts and Immunoblotting

After indicated treatments, cells were lysed in 1× lysis buffer (Cell signaling). Equal amount of total protein were subjected to SDS-PAGE gel, transferred to nitrocellulose, and then immunoreacted with primary antibody, followed by secondary antibody.

2.4. Co-Immunoprecipitation

Equal amounts of protein were incubated with primary antibody for overnight at 4 °C, followed by adding protein A&G beads for additional 2 h in room temperature. The beads were precipitated by centrifugation at 1000*g* for 2 min, and then were rinsed with PBS for 3 times. Proteins on the beads were eluted by boiling in SDS sample buffer.

2.5. Immunostaining

MLE12 cells were cultured in glass-bottom dishes and fixed with 3.7% formaldehyde for 20 min. Permeabilization in 0.1% Triton-100 for 1 min was performed for determining localization of LPA1-V5, LPA1-myc, HA-Nedd4L, or USP11-V5. Cells were exposed to primary antibody, followed by incubation with fluorescence-labeled secondary antibody. Immunofluorescent cell imaging was performed using a Zeiss LSM 510 confocal microscope.

2.6. Reverse Transcription (RT) Realtime PCR

Cells were collected after indicated treatment, and then total RNA was extracted using Trizol reagent from Life Technologies. 1 μg of RNA was used for reverse transcription reaction to generate cDNA. Realtime PCR was performed using Bio-Rad Ssofast Evagreen supermix reagent with synthesized cDNA as template. PCR primers were designed for detecting human IL-8, IL-6, and mouse KC gene.

2.7. Animals

C57/BL6 mice (6–8/group) were given intratracheal (i.t.) LPS (2 mg/kg body weight) for 24 h. BAL fluid was collected for cytokine analysis using ELISA. Mouse *Usp11* shRNA was inserted into a pLVX-IRES vector (Clontech); Lenti-shUSP11 viral and control viral vectors were generated by using a lentivirus packaging system (Clontech). C57/BL6 mice were given i.t. Lenti-control or Lenti-USP11 shRNA (10^9 plaque-forming units/mouse) for 7 days prior to i.t. inoculation with LPS (2 mg/kg weight) for 24 h. BAL fluid was collected for cytokine assays and lung tissues were fixed for hematoxylin and eosin (H&E) staining. To determine the effect of MX on lung inflammation, C57/BL6 were given i.t. MX (0.25 mg/kg body weight) prior to LPS challenge, and then BAL fluids and lung tissues were randomly and blindly analyzed as described above. All animal procedures in this study were performed in adherence with the National Institute of Health Guidelines on the use of Laboratory Animals and have been approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

2.8. Statistical Analysis

All results were subjected to statistical analysis using Microsoft Excel or ANOVA, and, wherever appropriate, the data were analyzed by Student's *t*-test and expressed as means ± SD. Data were collected from at least three independent experiments, and $p < 0.05$ was considered significant.

3. Results

3.1. Site Specific Ubiquitination of LPA1 Promotes LPA1 Degradation

Ligand-induced receptor degradation plays a negative feedback loop to attenuate membrane receptor signaling. To investigate the molecular regulation of LPA1 degradation, murine lung epithelial (MLE12) cells were treated with LPA, and protein levels of endogenous and over-expressed V5-tagged LPA1 (LPA1-V5) were examined by immunoblotting. As shown in Fig. 1a and b, LPA treatment reduces LPA1 levels in a time dependent manner. As ubiquitination is a recognized signal for receptor degradation, we examined the whether LPA1 is ubiquitinated and its role in LPA1 degradation. LPA1 ubiquitination was examined by immunoprecipitation with an ubiquitin antibody, followed by LPA1 immunoblotting. LPA treatment induced mono-ubiquitination of LPA1 in 30 min (Fig. 1c). To determine whether LPA1 is degraded in the lysosome, the localization of LPA1-V5 was determined by immunofluorescence staining. LPA1-V5 was co-localized with lysosome marker after LPA treatment (Fig. 1d). Further, inhibition of lysosome function by leupeptin or bafilomycin A1 impaired LPA-induced LPA1-V5

Download English Version:

https://daneshyari.com/en/article/2120631

Download Persian Version:

https://daneshyari.com/article/2120631

Daneshyari.com