Long noncoding RNA HAGLROS regulates cell apoptosis and autophagy in lipopolysaccharides-induced WI-38 cells via modulating miR-100/NF-κB axis

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Abstract
Pneumonia is a lower respiratory disease caused by pathogens or other factors. This study aimed to explore the roles and mechanism of long noncoding RNA HAGLROS in lipopolysaccharides (LPS)-induced inflammatory injury in pneumonia. The HAGLROS expression in serum of patients with acute stage pneumonia was detected. To induce pulmonary injury, WI-38 human lung fibroblasts were stimulated with lipopolysaccharides (LPS). The HAGLROS expressions in LPS-treated WI-38 cells and the effects of HAGLROS knockdown on the viability, apoptosis, and autophagy of LPS-induced cells were detected. Moreover, the regulatory relationship between HAGLROS and miR-100 was explored as well as the functional targets of miR-100 were identified. Furthermore, the regulatory relationship between miR-100 and PI3K/AKT/NF-κB pathway was elucidated. LncRNA HAGLROS was higher expressed in serum of patients with acute stage pneumonia compared with that in serum of healthy control. LPS caused WI-38 cell injury and increased HAGLROS levels. Downregulation of HAGLROS alleviated LPS-induced cell injury via increasing cell viability, and inhibiting apoptosis and autophagy. Moreover, there was a negative correlation between HAGLROS and miR-100, and the effects of HAGLROS downregulation on LPS-induced apoptosis and autophagy in WI-38 cells were by regulation of miR-100. Furthermore, NFκB3 was verified as a functional target of miR-100 and effects of miR-100 inhibition on LPS-induced WI-38 cell injury were alleviated by knockdown of NFκB3. Besides, Knockdown of HAGLROS inhibited the activation of PI3K/AKT/NF-κB pathway. Our findings reveal that downregulation of HAGLROS may alleviate LPS-induced inflammatory injury in WI-38 cells via modulating miR-100/NF-κB axis. HAGLROS/miR-100/NF-κB axis may provide a new strategy for treating acute stage of pneumonia. © 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Pneumonia is a lower respiratory disease caused by pathogens or other factors. The typical symptoms of pneumonia are dry cough, chest pain, fever, shortness of breath and fixed wet lung sound [1,2]. Moreover, pneumonia represents a major cause of death, especially in children and elderly people [3,4]. Community-acquired pneumonia, a common type of pneumonia, is responsible for high rates of morbidity and mortality worldwide [5]. Therefore, it is urgent to elucidate the key molecular mechanism underlying pneumonia, thus improving the clinical outcomes of this disease.

Accumulating evidences have disclosed several molecular mechanisms underlying pneumonia. For instance, high expression levels of interleukin-10 and interferon-induced protein 10 are associated with more severe hypoxic pneumonia in human immunodeficiency virus (HIV)-infected infants [6]. Four risk single-nucleotide polymorphisms (SNPs) located in chromosomes 1 and 17 contribute to the development of severe pneumonia in A/H1N1 infection [7]. Moreover, RNA-sequencing analysis has identified several miRNAs (hsa-let-7f-1, hsa-miR-455 and hsa-miR-200b) that may play a role in pneumonia pathogenesis [8]. However, the association of long noncoding RNAs (lncRNAs) with pneumonia has not been fully investigated.

LncRNAs are transcribed RNAs with length more than 200
nucleotides, which are not translated into proteins [9]. Growing
evidences link the dysregulation of IncRNAs to the pathophysio-
logical development of inflammatory diseases, such as inflamma-
tory bowel disease [10], rheumatoid arthritis and osteoarthritis
[11], as well as lung diseases, including pulmonary fibrosis [12] and
lung cancer [13]. Although a recent IncRNA sequencing analysis has
identified the potential Key Long Non-Coding IncRNAs associated
with pneumonia [14], the crucial IncRNAs that are correlated with
pneumonia development are largely unknown. HAGLROS is a 699
bp IncRNA that has recently been found to promote the malignant
development of in

3.6 years; 15 males and 5 males) were also
recruited as controls. A total of 3 mL of fasting peripheral venous
blood was collected from all enrolled individuals. Followed by
centrifugation under 2000 r/min, serum was collected and then
serum (FBS; Gibco, Grand Island, NY, USA), 50
μg/ml of g/ml E.coli
derived lipopolysaccharide (LPS) from strains 026:B6 (Sigma-
Aldrich) were added to treat different transfected cells, and cells
without LPS treatment were used as blank control. After 24, 48, 72 h
of LPS treatment, cell suspension was collected for further analyses.

2.4. Real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen). Approximately 2 μg of total RNA was reverse-transcribed into cDNA
using an M-MLV Reverse Transcriptase kit (Invitrogen). Relative
gene expression was analyzed by real-time qRT-PCR using a stan-
ard SYBR Green PCR kit (Toyobo, Osaka, Japan). U6 and β-actin
were used as references for miRNAs and mRNAs, respectively. The
relative quantitation of gene expression was determined using 2−ΔΔCT method. Each sample was analyzed in triplicate.

2.5. Vector construction and dual-luciferase reporter assay

The 3’-UTR of NFKB3 containing miR-100 binding site (NFKB3-
WT) was synthesized by Invitrogen (Shanghai, China) and then
inserted downstream of the luciferase reporter in the pmirGLO
Dual-Luciferase miRNA Target Expression Vector (Promega, Madi-
son, USA). The identical flanking nucleotides of NFKB3 3’-UTR
containing the mutated miR-100 target sequence (NFKB3-MUT)
was used as the control. For dual-luciferase reporter assay, WI-
38 cells were plated in 24-well plates and transfected with miR-100
mimic or mimic control and with the above constructed luciferase
vectors. After 48 h transfection, cells were harvested for measuring
the luciferase activity using the Dual-Glo luciferase assay kit
(Promega).

2.6. Cell viability assay

MTT assay was applied for determining cell viability of different
groups. Briefly, approximately 2 × 103 WI-38 cells were plated in a
96-well plate and incubated overnight. At various times following
different treatment, the medium was removed, and 20 μl MTT
(5 mg/ml, Sigma-Aldrich) was added to incubate cells for 4 h at
37 °C. The harvested formazan precipitates following centrifuga-
tion were then dissolved in 150 μl of dimethyl sulfoxide. The
absorbance of each well at 470 nm was then detected using a MRX II
absorbance reader (DYXEN Technologies, Chantilly, Virginia, USA).

2.7. Detection of cell apoptosis

Following different treatment, 1 × 103 cells were harvested,
washed twice with pre-chilled PBS and resuspended in 100 μl
binding buffer. According to the manufacturer’s protocol of
Annexin V–FITC Apoptosis Detection Kit (BD Biosciences, San Jose,
CA, USA), double-staining of Annexin V and propidium iodide (PI)
was then performed. Cell apoptosis was the detected by flow
cytometry within 1 h by means of the BD LSRII Flow Cytometer
System with FACSDiva Software.

2.8. Western blot

Different treated cells were collected and lysed with cell lysis
buffer (Beyotime, Haimen, China) for extraction of total protein.
Followed by determining the protein concentration by Bradford
reagent (Biorad laboratories, CA, USA), protein extracts (30 μg per
lane) were separated on 12% SDS-polyacrylamide gels and trans-
ferred onto polyvinylidene fluoride (PVDF) membranes (Millipore,
Billerica, MA, USA). By incubation with primary antibodies to
against LC3I, LC3II, Beclin1, NFKB3, p-NFKB3, p-P38K, P38K, AKT, p-
AKT, Bcl-2, Bax, caspase-3, caspase-9, and β-actin (1:1000, Santa
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