International Immunopharmacology 59 (2018) 252–260

Contents lists available at ScienceDirect

International Immunopharmacology

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

Long non-coding RNA NEAT1 plays an important role in sepsis-induced acute kidney injury by targeting miR-204 and modulating the NF-κB pathway

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ARTICLE INFO

Keywords:
Sepsis
Acute kidney injury
NEAT1
MiR-204
NF-κB pathway

ABSTRACT

This study aimed to explore the role of long non-coding RNA NEAT1 in sepsis-induced acute kidney injury (AKI). The expression levels of NEAT1 in sepsis-induced AKI patients were detected. The rat mesangial cells (RMCs) were treated with lipopolysaccharide (LPS) to induce cell injury. Then, the effects of NEAT1 suppression on the cell viability, apoptosis, cytokines expression, and oxidative stress in the LPS-stimulated RMCs were tested. The regulatory miRNA of NEAT1, as well as the target genes of this miRNA, were investigated. Moreover, the regulatory relationship between NEAT1 and the NF-κB pathway was explored. The results demonstrated that NEAT1 was significantly upregulated in the sepsis-induced AKI patients. Moreover, the upregulation of NEAT1 was associated with the serious degrees of AKI in sepsis patients. In addition, the suppression of NEAT1 alleviated LPS-induced injury in RMCs. MiR-204 was negatively regulated by NEAT1. Suppression of NEAT1 alleviated LPS-induced injury by overexpression of miR-204. Moreover, IL-6R was a target of miR-204, and the effects of the suppression of NEAT1 on LPS-induced cell injury were caused by inactivating the NF-κB pathway. In conclusion, upregulation of NEAT1 may aggravate the LPS-induced injury by targeting miR-204 and activating the NF-κB pathway. NEAT1 may serve as an important diagnostic marker and therapeutic target in sepsis-induced AKI.

1. Introduction

Sepsis is a life-threatening condition arising when the body's response to infection causes injury to its own tissues and organs [1]. It is almost always associated with multiple organ failure [2]. Acute kidney injury (AKI) is a common and potentially fatal complication that occurs during the development of sepsis. It has been estimated that severe sepsis is the trigger for about 50% of the cases of AKI [3]. Sepsis-induced AKI is a serious medical problem in the intensive care unit, which is implicated in approximately 50% to 70% of mortality, depending on its severity [4,5]. However, limited understanding of the pathophysiological mechanisms involved in septic AKI has hindered the development of effective therapies.

In recent years, increasing attention has been given to the non-coding RNAs which play important roles in various biological processes, such as innate immunity and apoptosis [6–8]. The long non-coding RNAs (lncRNAs) comprise > 200 nucleotides, representing another group of transcripts. The mechanisms of lncRNAs in health and diseases have been comprehensively reviewed [22,23]. Recently, several studies reported the differential expression of lncRNAs in human cardiomyocytes, tubular epithelial cells, and monocytes after exposure to lipopolysaccharide (LPS) or the plasma of septic patients [9,10]. LncRNA NEAT1 is an essential architectural component of paraspeckle structure [11,12], and has recently been recognized to play an important role in the innate immune response [13]. However, to our knowledge, its role in sepsis-induced AKI has not been investigated.

In the present study, we aimed to explore the role of NEAT1 in sepsis-induced AKI. In order to achieve this, we analyzed the expression

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https://doi.org/10.1016/j.intimp.2018.03.023
Received 8 October 2017; Received in revised form 9 February 2018; Accepted 20 March 2018

levels of NEAT1 in the serum of sepsis-induced AKI patients. Subsequently, we explored the molecular mechanisms involved in the abnormal expression of NEAT1 in sepsis-induced AKI through the establishment of in vitro cell experiments. The findings of this study may contribute to the development of novel therapeutic approaches for sepsis-induced AKI.

2. Materials and methods

2.1. Study population

In the time period between March 2015 and May 2017, 55 sepsis patients with different degrees of AKI were recruited in the study. According to the Acute Kidney Injury Network (AKIN) criteria [14], the patients were divided into non-AKI (NAKI, n = 16), AKI I (n = 13), AKI II (n = 11), and AKI III (n = 15) groups. In addition, 20 healthy volunteers were included as healthy controls. The diagnosis of sepsis was based on a presmend or identified source of infection combined with two or more of the following symptoms of the systemic inflammatory response syndrome: temperature < 36 °C or > 38 °C; respiratory rate > 20 breaths/min or hyperventilation with $PaCO_2$ < 32 mmHg; pulse rate > 90 beats/min; and white blood cell count > 10% immature cells, or > 12,000/mL, or < 4000/mL. Patients who were pregnant, under corticosteroids, bone marrow or organ transplant recipients, or diagnosed with acquired immune deficiency syndrome were excluded from the study. The clinical features of the enrolled subjects are listed in Table 1. All the patients provided an informed consent before the study. All procedures in this study were approved by the Protection of Human Ethics Committee of our hospital.

2.2. Detection of NEAT1 in the serum of subjects

Serum samples were obtained by centrifuging the blood samples from the subjects at 5000*g* for 3 min. Total RNA was isolated from serum using the miRNeasy Mini kit (QIAGEN, Germany). The cDNA synthesis was then performed using RT^2 PreAMP cDNA Synthesis Kit (QIAGEN, Germany). In order to detect the expression of NEAT1 in serum, quantitative reverse transcriptase (qRT)-PCR was performed using RT^2 SYBR® Green qPCR MasterMix (QIAGEN, Germany) on the StepOnePlus thermocycler (Applied Biosystems). The expression of NEAT1 was then calculated using the $2^{-\Delta\Delta in}$ method. Moreover, to detect the effects of NEAT1 on the pathology of sepsis-induced AKI, the correlation between serum NEAT1 expression and Creatinine clearance rate (CCr) levels was analyzed using Pearson's correlation coefficient in SPSS 19.0 (SPSS, Chicago, IL).

2.3. Cell culture and reagents

A rat mesangial cell line (RMC) was purchased from the American Type Culture Collection (Manassas, USA), and maintained in Dulbecco's modified Eagle's medium supplemented with 10%–15% fetal bovine serum. At about 60% confluence, the cells were treated with 100 ng/mL LPS (Sigma-Aldrich Inc., USA).

2.4. Lentiviral vector construction and infection

A short-hairpin RNA directed against NEAT1 (sh-NEAT1) was ligated into LV10-CMV-RFP-Puro vector (GenePharma, Shanghai, China). The lentiviral vector that included negative sequences (sh-NC) was used as negative control. The lentiviral expression vectors were packaged in 293T cells, and harvested after 72 h. Then, the cells were infected with virus particles with the help of 8 μg/mL Polybrene (Sigma, St. Louis, Missouri, USA), followed by selection with puromycin for up to seven days.

Furthermore, the complementary DNA (cDNA) encoding NEAT1 was amplified using the PCR based on PfuUltra II Fusion H DNA Polymerase (Stratagene, Agilent Technologies, Santa Clara, CA, USA), and subcloned into the *Hin*dIII and *Eco*RI sites of pcDNA3.1 vector (Invitrogen, USA); it was named pcDNA-NEAT1. si-IL-6R, si-NC, miR-204 mimic, mimic NC, miR-204 inhibitor, and inhibitor NC were purchased from GenePharma (Shanghai, China). Transfection of cells was performed using Lipofectamine 2000 (Life Technologies Corporation, Carlsbad, CA).

2.5. MTT assay

RMCs were seeded into 96-well plates at a density of 10,000 cells/well. After 12 h of incubation, the cells were transfected for 24 h in the presence of 0 or 100 ng/mL of LPS and then washed three times with PBS. Afterward, 500 μg/mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, USA) was pipetted into each well, and the plate was incubated for 3 h. After adding 200 μL of dimethyl sulfoxide solution (Sigma, USA), absorbance was measured at 570 nm using a microplate reader (Bio-Rad Model 550, Hercules, CA, USA).

2.6. Cell apoptosis

RMCs were seeded in 96-well plates at a density of 10,000 cells per well for 12 h. The cells were transfected in the presence of 100 ng/mL LPS or without the presence of LPS for 24 h. Then, the cells were double stained with FITC-Annexin V and propidium iodide (PI) using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences, USA), followed by an analysis using a flow cytometer (FACScan, BD Biosciences) equipped with CellQuest software (BD Biosciences). The cells were discriminated into viable cells, early apoptotic cells, apoptotic cells, and dead cells. The relative ratio of early apoptotic cells was compared with that of the control transfectants for each experiment.

2.7. Analysis of inflammatory cytokines

The concentrations of TNF-α, IL-6, IL-8, and IL-1β in the cell suspension were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Lucigenin assay for superoxide measurement

About 10,000 RMCs were incubated in control and LPS-containing

Table 1
The clinical features of the enrolled subjects.

Characteristics	Control	NAKI	AKI I	AKI II	AKI III
Number of patients	20	16	13	11	15
Sex (male/female)	13/7	11/5	9/4	7/4	10/5
Age (range in years)	35–65	38–72	36–76	32–69	30–70
Ccr (mL/min 1.73 m^2)	83.6 ± 13.8	73.2 ± 16.3	42.2 ± 11.4	30.2 ± 7.3	14.8 ± 6.7

Ccr, Creatinine clearance rate.