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MicroRNA-30b promotes lipopolysaccharide-induced inflammatory injury and alleviates autophagy through JNK and NF-κB pathways in HK-2 cells

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ABSTRACT

Background: Acute kidney injury (AKI) is an abrupt loss of kidney function. MicroRNA-30b (miR-30b) has been reported to be involved in the inflammatory reaction of a variety of diseases. However, the role of miR-30b in AKI remains unknown. In this research, we aimed to investigate the role of miR-30b in lipopolysaccharide (LPS)-induced kindey inflammatory injury *in vitro* and *in vivo*.

Methods: In vitro, after miR-30b mimic/inhibitor transfection and/or LPS treatment, the viability, apoptosis, autophagy and inflammatory cytokines releases, as well as activation of c-Jun-N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) pathways were detected by cell counting kit-8 (CCK-8) assay, flow cytometry, qRT-PCR, enzyme-linked immunosorbent assay (ELISA) and western blot, respectively. *In vivo*, after LPS treatment and/or anti-miR-30b administration, the levels of creatinine, the activities of alanine aminotransferase (ALT) and histologic scores, as well as concentrations of inflammatory cytokines were assessed by creatinine assay kit, ALT assay kit and ELISA, respectively.

Results: LPS inhibited HK-2 cell viability and induced HK-2 cell apoptosis, autophagy and the releases of inflammatory cytokines. Overexpression of miR-30b promoted LPS-induced HK-2 cell viability inhibition, cell inflammatory cytokines releases, cell apoptosis induction and activation of JNK and NF-κB signaling pathways, but inhibited LPS-induced HK-2 cell autophagy. Suppression of miR-30b had opposite effects. Moreover, suppression of miR-30b alleviated the LPS-induced kidney injury in mice model by decreasing creatinine level, ALT activity and histologic scores, as well as concentrations of inflammatory cytokines.

Conclusion: miR-30b participated in the LPS-induced kindey inflammatory injury *in vitro* and *in vivo*.

1. Introduction

Acute kidney injury (AKI) is an abrupt loss of kidney function [1]. AKI is a common complication in hospitalized patients, which affects 7% of patients in hospital and approximately 25–30% of patients in intensive care unit (ICU) [2]. Moreover, the mortality of AKI patients is very high [3]. Inflammation is now recognized to play a major role in the pathogenesis of AKI [4,5]. When kidney is injured, proximal tubular epithelial cells product inflammatory cytokines (including TNF-α, IL-1β and IL-6), and infiltrate into the interstitium of kidney. Therefore, a better understanding of the inflammatory response in AKI will be helpful for AKI treatment and improving patient's survival.

Programmed cell death pathways, including apoptosis and autophagy, are required for normal cell turnover and tissue homeostasis [6]. Unlike apoptosis, autophagy is a controlled program of degradation, recycling of cellular components, such as damaged macromolecules and organelles [7,8]. This process involves sequestration of macromolecules and cell organelles in autophagosomes [9]. The formation of an autophagosome is initiated by several autophagy genes including LC3, BECN1 (encoding Beclin-1 protein), and a network of autophagy-related gene (ATG) genes [9,10]. In response to external stress signals, autophagy plays a critical role in cellular survival rather than cell death [11,12]. Previous studies have been reported that autophagy is reported to be a reno-protective mechanism in ischemia-reperfusion injury [13,14] and cisplatin injury [14,15].

microRNAs (miRNAs) are short non-coding RNAs with 20–24 nucleotides in length, which have been widely reported to post-transcriptionally regulate gene expression by binding to the 3′untranslated regions (3′UTR) of target mRNAs [16]. Recent evidences showed that miRNAs played important roles in inflammatory diseases [17,18]. miRNA-30b (miR-30b) was showed to contribute to inflammatory cytokine-mediated β-cell dysfunction [19] and involved in

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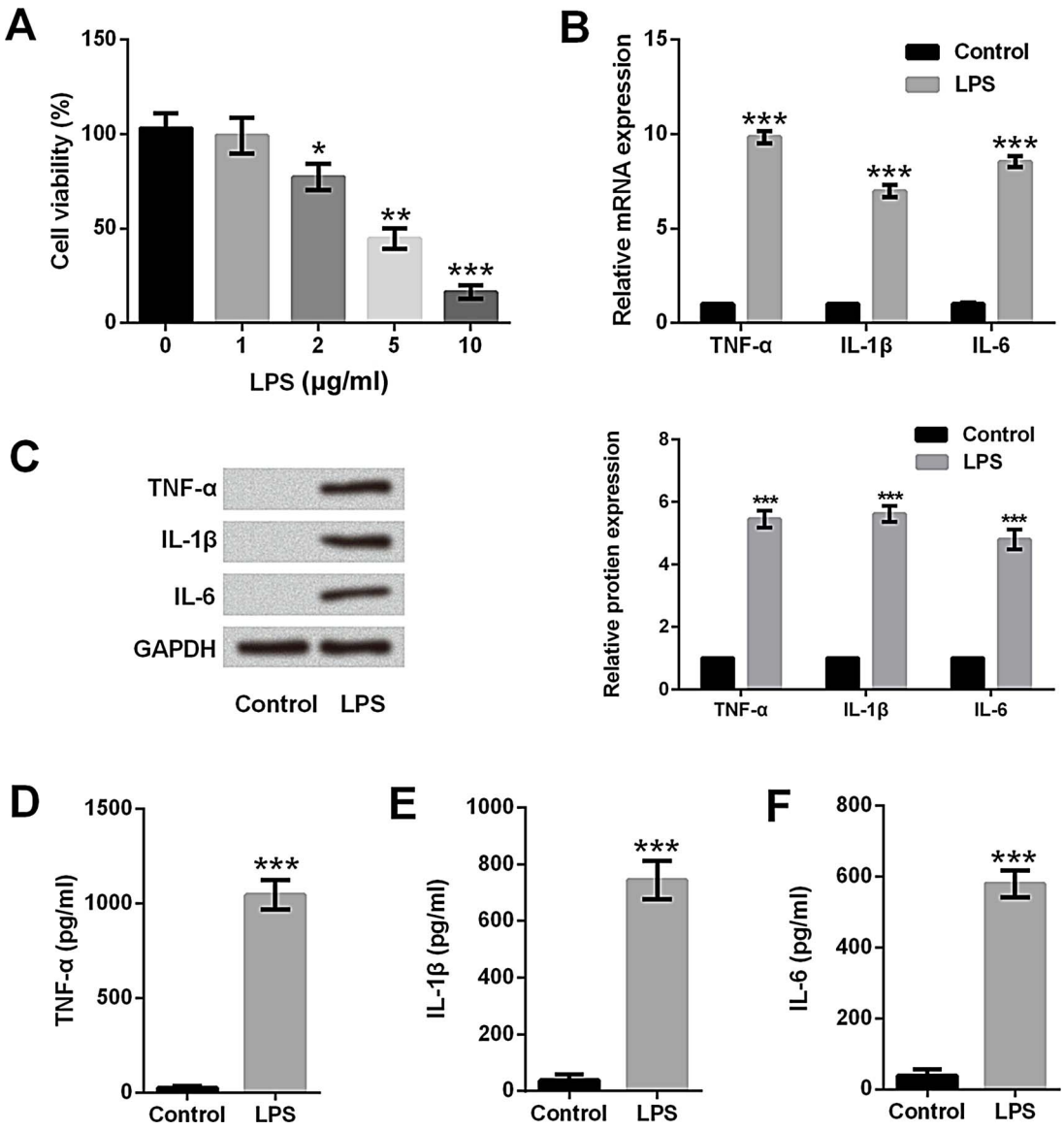

Fig. 1. LPS inhibited HK-2 cell viability and induced the releases of inflammatory cytokines. (A) After 1, 2, 5 or 10 μg/ml LPS treatment for 5 h, the viability of HK-2 cells was assessed by cell counting kit-8 (CCK-8) assay. (B) After 5 μg/ml LPS stimulation for 5 h, the mRNA levels of TNF-α, IL-1β and IL-6 were detected by quantitative reverse-transcriptase PCR (qRT-PCR). (C) The protein expressions of TNF-α, IL-1β and IL-6 in LPS-stimulated HK-2 cells were detected by western blot. (D–F) The concentrations of TNF-α, IL-1β and IL-6 in culture supernatant of LPS-stimulated HK-2 cells were detected by enzyme-linked immunosorbent assay (ELISA). LPS: Lipopolysaccharide, TNF-α: Tumor necrosis factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6. All experiments were repeated three times (N = 3). One-way analysis of variance (ANOVA) was used to calculate P-values. $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$ *vs.* Control.

immunosuppression of melanoma cells [20]. Also, miR-30b was reported to inhibit autophagy in hepatic ischemia-reperfusion injury [21] and cancer cells [22]. However, the role of miR-30b in kidney injury remains unknown.

c-Jun-N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) pathways have been reported to exert key roles in the regulation of cell inflammatory reaction, cell apoptosis and cell autophagy [23,24]. Joo et al. proved that dipeptidyl peptidase IV inhibitor alleviated AKI in rat kidney injury by inactivating JNK pathway [25]. Yu et al. demonstrated that Rhein prevented endotoxin-induced AKI by inhibiting NF-κB pathway [26].

In the present study, lipopolysaccharide (LPS) was used to treat human proximal tubular epithelial cells (HK-2 cells) and mice to establish inflammatory injury model of kidney *in vitro* and *in vivo*. Then, the roles of miR-30b in LPS-stimulated HK-2 cell viability inhibition, inflammatory cytokines releases, apoptosis induction, as well as autophagy enhancement were investigated *in vitro*. We found that miR-30b promoted LPS-induced HK-2 cell viability inhibition, releases of inflammatory cytokines and apoptosis, but alleviated cell autophagy probably through activating JNK and NF-κB signaling pathways. Moreover, we also found that suppression of miR-30b alleviated the LPS-induced kidney injury in mice model.

2. Materials and methods

2.1. Cell culture and treatment

Human proximal tubular epithelial cell line HK-2 was purchased from the American Type Culture Collection (Catalogue No.: CRL-2190™; ATCC, Manassas, VA, USA). HK-2 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Invitrogen), penicillin (100 U/ml, Invitrogen), and streptomycin (100 μg/ml, Invitrogen). Cultures were maintained at 37 °C in a