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microRNA-300/NAMPT regulates inflammatory responses through activation of AMPK/mTOR signaling pathway in neonatal sepsis

Yexuzi Li[a,1], Junzhong Ke[a,1], Chen Peng[a], Fugen Wu[b,*], Yukang Song[a,*]

[a] Department of Medical Intensive Care Unit, The First People's Hospital of Wenling, Wenling 317500, Zhejiang Province, China
[b] Department of Neonatal Intensive Care Unit, The First People's Hospital of Wenling, Wenling 317500, Zhejiang Province, China

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ABSTRACT

Aim: Rapid and accurate diagnosis of neonatal sepsis (NS) is highly warranted because of high associated morbidity and mortality. The study aims to evaluate the effects of miR-300 on inflammatory responses in a septic neonate mouse model.

Methods: A septic mouse model was established by intraperitoneal (i.p.) cecal slurry (CS) injection in order to validate the effect of miR-300 on the inflammatory response in endothelial cells. Bioinformatics tools and luciferase activity were employed to detect the target of miR-300. Serum inflammatory factors were determined by ELISA assay. RT-qPCR and western blot analysis were used to determine the gene expressions. Flow cytometry was employed to evaluate cell apoptosis.

Results: Gain- and loss-of-function studies revealed that miR-300 overexpression augmented autophagy, inhibited cell apoptosis, enhanced cell cycle entry in endothelial cells, and decreased inflammatory response through the regulation of pro- and anti-apoptotic factors in endothelial cells. The effect of miR-300on endothelial cells was upregulated after nicotinamide phosphoribosyltransferase (NAMPT) silencing and AMPK/mTOR signaling pathway activation, indicating that miR-300 influences sepsis via suppressing NAMPT and triggering the AMPK/mTOR signaling pathway.

Conclusions: Our study provides evidence indicating that overexpressedmiR-300 enhances autophagy by targeting NAMPT through activation of the AMPK/mTOR signaling pathway in septic mouse models, indicating it may serve as a potential therapeutic target for sepsis.

1. Introduction

Sepsis is defined as the response disorders of the host to infection, resulting in life-threatening organ dysfunction [1]. Sepsis remains the main cause of high morbidity and mortality rates worldwide, with the highest incidence in the elderly population [2]. In fact, sepsis is the third leading cause of death during the neonatal period (from birth to 28 days). The incidence of sepsis is alarmingly high in very-low-birth-weight infants (those weighing < 1500 g) [3]. The incidence of neonatal sepsis (NS) is estimated to be 25–30% and the death rate is high up to 52% [4]. Although tremendous efforts have been made in the diagnosis and treatment, sepsis is still a major medical and health care problem. It is a challenging task to explore effective medicines in the treatment of sepsis. Recent studies have demonstrated that microRNA (miRNAs) is a class of endogenous, non-encoding small molecules (~22 nt), which are transcribed in the cell nucleus and transported into the cell cytoplasm [5]. miRNA inhibits the translation or degradation of target mRNA and exerts negative regulatory effect on gene expression through the interaction with 3′-UTR of target mRNA. At present, approximately 2,600 types of miRNAs have been identified in human, which may directly regulate at least 30% of the genes in cells [6]. Therefore, miRNA probably participates in regulating almost all major cell functions including cell development, differentiation, proliferation and apoptosis [7]. Currently, multiple miRNAs, such as miRNA-146a, miRNA-499-5p, miRNA-297, miRNA-574-5p, miRNA-122 and miRNA-133a, have been confirmed to be dysregulated in human sepsis, which provide reference value for the clinical diagnosis and prognosis of sepsis [8–12]. Nevertheless, whether miRNA-300 is correlated with NS remains elusive.

* Corresponding authors at: NO. 333, Chuan'an Nan Street, Chengxi District, Wenling 317500, Taizhou, Zhejiang Province, China
E-mail addresses: yexuzili@163.com (Y. Li), kejunzhongdoc@sina.com (J. Ke), linling_jiang@163.com (C. Peng), wufu_gen@163.com (F. Wu), garysongyk@163.com (Y. Song).
[1] The first two authors should be regarded as joint First Authors.

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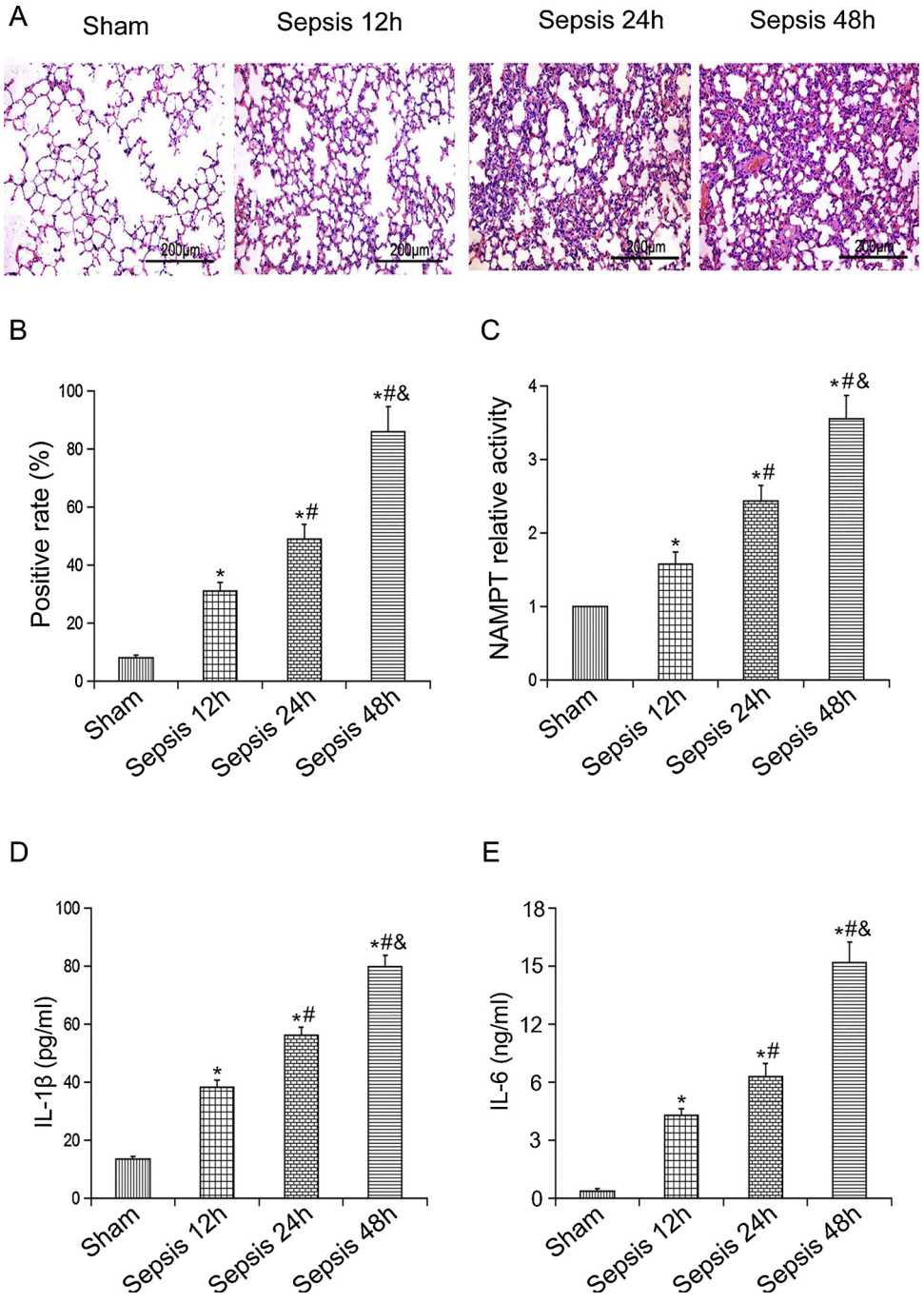

Fig. 1. Immunohistochemical staining for pathological observation and detection of NAMPT expression in lung tissues. The expression of NAMPT was up-regulated after the sepsis model was established (400×, A) and in the sepsis group (B); the activity of NAMPT in the sepsis group was significantly higher than that in sham operation group; C, The levels of IL-1β (D) and IL-6 (E) were analyzed by ELISA. *p < 0.05 vs. the Sham group; #p < 0.05 vs. the sepsis 12 h group; &, p < 0.05 vs. the sepsis 24 h group. Three replicates per group, three independent experiments per group.

2. Materials and methods

2.1. Cell culture

HEK293T cells were grown in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT), supplemented with 10% fetal bovine serum and incubated at 37 °C in a humidified incubator with 5% CO2.CD1 mouse primary pulmonary vein endothelial cells were isolated from pulmonary vein tissue of mice. CD1 cells were grown in T25 tissue culture flasks pre-coated with gelatin-based coating solution for 2 min and incubated in Cell Biologics'Culture Complete Growth Medium generally for 3–7 days.

2.2. Ethics statement

This study was carried out in strict accordance with the guidelines of the Laboratory Animal Care and Use Committee of Zhejiang Province in China. The research procedures have been approved by the Animal Ethics Committee of the First People's Hospital of Wenling. All efforts have been made to reduce the pain of experimental animals as possible.