Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MiR-107 induces TNF-α secretion in endothelial cells causing tubular cell injury in patients with septic acute kidney injury

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ARTICLE INFO

Article history:
Received 22 December 2016
Accepted 3 January 2017
Available online xxx

Keywords:
Sepsis
Acute kidney injury
Endothelial cells
miRNA

ABSTRACT

Activation of endothelial cells plays a key role in septic acute kidney injury (AKI). This study investigated the role of miRNA in endothelial-induced tubular cell injury in sepsis. Circulating endothelial cells (CECs) from septic AKI, non-septic AKI, septic non-AKI patients and healthy volunteers were isolated and cultured, and HK2 cells were exposed to CEC-conditioned medium. CEC-conditioned medium prepared from septic AKI patients led to cell shrinkage, decreased E-cadherin, the release of NAG and cell apoptosis in HK2 cells. TNF-α mediated the tubular cell injury induced by CEC-conditioned medium prepared from septic AKI patients. PCR array analysis detected that miR-107 was significantly increased in the CECs of septic AKI patients. MiR-107 was verified to target the 3′UTR of Dual-specificity phosphatase 7(DUSP7). Transfection of miR-107 ASO recovered the expression of DUSP7, suppressed the phosphorylation of ERK, and decreased the secretion of TNF-α in the CECs of septic AKI patients and in the peritubular endothelial cells of septic AKI mice. The inhibition of miR-107 prevented the decrease of E-cadherin, the release of NAG and cell apoptosis in HK2 cells exposed to CEC-conditioned medium prepared from septic AKI patients, and preserved the normal renal morphology and decreased the serum creatinine level in septic AKI mice. In conclusion, our study suggests that the increased miR-107 induces TNF-α secretion by targeting DUSP7 in endothelial cells, which may directly cause tubular cell injury in septic AKI.

1. Introduction

The development of acute kidney injury (AKI) is one of the most frequent complications of sepsis, which increases the complexity and cost of care. The presence of shock, low white blood cell counts and low platelet counts are risk factors [1,2]. Previous studies highlighted systemic hypotension, renal vasoconstriction and ischemia reperfusion injury as the pathophysiological mechanisms of septic AKI. However, this view has been challenged by the fact that septic AKI occurs in the context of renal vasodilatation and increased renal blood flow [3]. Currently, inflammatory apoptosis is considered to be the culprit in septic AKI [4]. This situation is different from the mechanism of non-septic AKI, which is caused by ischemia, urinary tract obstruction or drug toxicity.

During septic AKI, activation of the endothelium leads to increased expression of endothelial adhesion molecules, which in turn leads to leukocyte activation, creating a vicious cycle in the inflammatory response [5]. In addition, a breakdown of endothelial barrier function occurs. The loss of fluid into the extravascular space leads to edema in the kidney [5]. Previous studies have shown that miRNAs participate in regulating endothelial barrier function and cell apoptosis in sepsis [6,7]. In this study, we assessed whether endothelial cells could directly cause tubular cell injury in septic AKI and the role of miRNA in the mechanism.

2. Materials and methods

2.1. Patients and control subjects

Septic AKI patients were recruited at the department of emergency during 2015–2016. Non-septic AKI patients, septic non-AKI patients and healthy volunteers served as controls (Table 1). Sepsis was diagnosed according to the "2005 International Pediatric Sepsis Consensus Conference" criteria [8], and AKI was diagnosed according to the KDIGO AKI criteria [9]. This study was approved by the ethics committees of Children's Hospital of Nanjing Medical University. Written informed consents were obtained from children's guardians. The research work was in compliance with the Helsinki Declaration.

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http://dx.doi.org/10.1016/j.bbrc.2017.01.013
0006-291X/

Table 1
Clinical features of enrolled septic AKI, non-septic AKI and septic non-AKI patients.

	Healthy volunteers	Septic AKI	Non-septic AKI	Septic non-AKI
Number	15	15	15	15
Age(months)	73.9 ± 11.2	67.0 ± 22.2	64.2 ± 21.5	71.0 ± 11.4
Sex(male:female)	8:7	9:6	8:7	9:6
PRISM III score		11.9 ± 4.0	6.5 ± 2.0#	6.6 ± 2.0#
Scr(mg/dl)	0.44 ± 0.09	2.03 ± 0.70*	1.91 ± 0.81*	0.48 ± 0.11#
Plasma TNF-α(pg/ml)	7.17 ± 1.74	195.28 ± 29.63*	31.01 ± 10.03*#	50.64 ± 16.96*#

PRISM III, Pediatric Risk of Mortality score; Scr, serum creatinine.
*$p < 0.05$, *versus* healthy volunteers; #$p < 0.05$, *versus* septic AKI patients.

2.2. Isolation of circulating endothelial cells

Isolation of circulating endothelial cells (CECs) was performed by immunomagnetic separation as previous report [10]. The endothelial nature of the isolated cells was confirmed by anti-CD31 staining. The purity of isolated CECs was generally more than 90%.

2.3. Preparation of CECs conditioned medium

As monoculture CECs failed in growth, a HUVECs feeder layer was used for co-culture of CECs to provide a permissive environment as previous report [11]. After 5 days, colonies of isolated cells were formed within the sparse feeder layer. The cells were washed and incubated with serum-free media for another 24 h. Then the CECs conditioned medium were collected and kept frozen at −70 °C until used.

2.4. Treatment of tubular cells

Human tubule epithelial (HK2) cells were grown in DMEM/F12 medium that contained 10% fetal bovine serum. Cells were serum starved overnight before treatments. CECs conditioned medium prepared from healthy volunteers, non-septic AKI or septic non-AKI patients were diluted 2-fold with serum-free DMEM/F12 medium, and CECs conditioned medium prepared from septic AKI patients were diluted 2-fold, 4-fold or 8-fold with serum-free DMEM/F12 medium.

In order to study the role of cytokines in tubular cell injury, HK2 cells were treated with TNF-α (150 pg/ml) with serum-free DMEM/F12 medium for 24 h, or incubated with antibodies against TNF-α (0.1 μg/ml), TGF-β (10μg/ml) and IL-1α (0.1 μg/ml) 1 h before stimulating with CECs conditioned medium prepared from septic AKI patients diluted 2-fold with serum-free DMEM/F12 medium for 24 h. Cell apoptosis was analyzed with Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, St. Louis, MO, USA).

2.5. RT-PCR analysis

The level of miR-107 was quantified with the TaqMan MicroRNA Cells-to-CT Kit (Applied Biosystems, Foster City, CA). RT-PCR analysis of E-cadherin was performed using specific primers (sense, 5′-CGAGAGCTACACGTTCACGG-3′; anti-sense, 5′-GTGTCGAGGGAAAAATAGGCTG-3′).

2.6. Western blot analysis

Western blots were performed with antibodies against E-cadherin, DUSP7, ERK1/2, p-ERK1/2 and β-actin (Santa Cruz, CA, USA).

2.7. Analysis of NAG release

N-acetyl-β-glucosaminidase (NAG) as a marker of tubular cell damage was assessed using assay kit (CS0780) purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.8. MiRNA PCR array analysis

After magnetic separation, isolated CECs from 1 ml blood of five patients were pooled and extracted using miRNeasy Mini Kit. MiRNAs from the CECs were converted to cDNA using the miRNA first-strand kit, and then detected using the Human miRNome miRNA PCR Array (SABiosciences, Frederick, MD).

2.9. Luciferase assays

The luciferase reporter gene assay was conducted as previous reports [12]. HUVECs were transiently cotransfected with 0.1 μg of the reporter constructs, 0.02 μg of the renilla construct and 50 nM miR-107 mimics or negative control mimics. The firefly and renilla luciferase activity were determined using the Dual-Luciferase Reporter Assay System (Promega).

2.10. Animals

Animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals, and approved by the Institutional Animal Care and Use Committee. Male C57BL/6J mice, 8–12 weeks of age, were used in this study. Mice were injected intraperitoneally with LPS (8 mg/kg) and killed at 20 h. To examine the effect of miR-107 inhibition, 50 μg of miR-107 antisense oligonucleotides (ASO) complexed with atelocollagen was administered intravenously into mice 15 min after LPS injection. Since mice underwent oligo- or anuria, urine analysis was not performed. Renal peritubular endothelial cells were isolated and cultured as previous report, to analysis the level of miR-107 and the secretion of TNF-α [13].

2.11. Statistical analysis

All of the data are expressed as the means ± s.d. The data from multiple groups were analyzed with a one-way analysis of variance followed by the Student–Newman–Keuls test. Data from two groups were compared by t-tests. *P*-values of <0.05 were considered significant.

3. Results

3.1. CEC-conditioned medium prepared from septic AKI patients causes tubular cell injury

CEC-conditioned medium prepared from septic AKI patients led to the loss of cobblestone appearance, cell shrinkage, and loss of cell-to-cell contact in HK2 cells in a dose-dependent manner, while CEC-conditioned medium prepared from healthy volunteers, non-

Please cite this article in press as: S. Wang, et al., MiR-107 induces TNF-α secretion in endothelial cells causing tubular cell injury in patients with septic acute kidney injury, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.01.013