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Original Article

MicroRNA-21 is upregulated during intestinal barrier dysfunction induced by ischemia reperfusion

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Abstract This study aimed to investigate the expression of miRNA-21 during intestinal barrier dysfunction induced by intestinal ischemia reperfusion. Forty SPF SD rats were divided into 5 groups randomly. Intestinal ischemia-reperfusion injury (IRI) was induced by mesenteric artery occlusion for 1 h and reperfusion for 1 h, and the rats were sacrificed at 1, 3, 6 and 12 h after reperfusion. Fresh intestine tissues were immediately isolated for the measurement of trans-epithelial electrical resistance (TER). The levels of cytokines, ICAM-1, DAO, iFABP and MPO in serum were determined by ELISA. Intestinal tight junction proteins occludin and claudin-1 were detected by immunofluorescence analysis and Western blot analysis. miR-21 expression in intestinal tissues was measured by RT-PCR. Compared with sham group, the levels of pro-inflammatory cytokines TNF-α and IL-6 and ICAM-1, DAO, iFABP and MPO increased while IL-10 level decreased in intestinal ischemia-reperfusion group. In addition, the levels of intestinal tight junction proteins occludin and claudin-1 decreased while miR-21 level increased in intestinal ischemia-reperfusion group, compared with sham group. In conclusion, miR-21 expression is upregulated during intestinal barrier dysfunction induced by IRI. miR-21 may play an important role in the regulation of intestinal barrier function.

Conflicts of interest: All authors declare no conflicts of interests.

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Introduction

Intestinal ischemia-reperfusion injury (IRI) frequently occurs during abdominal surgery, small bowel transplantation and hemorrhagic shock [1]. IRI is associated with oxidative stress with subsequent inflammatory injury that can lead to systemic inflammatory response syndrome (SIRS) and even life-threatening multiple organ dysfunction (MODS), thus IRI has high morbidity and mortality [2,3]. The gut contains a large number of endotoxins and pathogens, but healthy intestinal barrier can effectively prevent endotoxin and intestinal bacteria from entering into the body. Intestinal mucosal barrier is composed of chemical barrier, mechanical barrier, immune barrier and biological barrier, and mechanical barrier is based on a complete connection between intestinal epithelial cells and forms the foundation of intestinal mucosal barrier. The injury of mucosal barrier is the most critical aspect of IRI. IRI causes damage to intestinal barrier function and systemic inflammatory response [4,5].

It has been widely recognized that a variety of factors such as ischemia-induced intestinal epithelial damage and apoptosis, increased permeability of epithelial cells, endotoxin and intestinal bacterial translocation are the main cause of IRI. In addition, intestinal infection induced by intestinal barrier dysfunction is a major cause of multiple organ failure and patients death. Therefore, further understanding of molecular mechanisms of IRI is necessary to develop therapeutic approach for IRI.

MicroRNAs are a class of about 22 nt long non-protein-coding single-stranded small RNA nucleotides involved in post-transcriptional regulation of gene expression and play an important role in apoptosis, cell proliferation and differentiation, metabolism and other processes [6]. Recent studies suggest the role of miRNAs in regulating intestinal tight junction permeability [7]. In particular, miR-21 was shown to be upregulated in the patients with ulcerative colitis and overexpression of miR-21 caused intestinal epithelial barrier impairment [8]. However, the role of miR-21 in IRI remains unclear. Therefore, this study aimed to investigate the expression change of miR-21 during IRI. We established IRI animal model in vivo and detected the expression of miR-21 and intestinal epithelial tight junction proteins in intestinal tissues.

Materials and methods

Animals

Adult male SD rats (weight 180—220 g, about 8 weeks old) were purchased from the Experimental Animal Center of Fudan University (Shanghai, China). The experiment protocol was approved by the Ethic Committee of Animal Care. The rats were maintained in cages at room temperature with 12 h light/dark cycle with free access to the food and water. The rats were anesthetized with ketamine and received midline laparotomy, then the intestine was externalized, and IRI was induced by clamping of superior mesenteric artery with microvascular clamps for 1 h (ischemia) followed by reperfusion for 1 h. Forty SPF SD rats were divided into 5 groups randomly ($n = 8$). In Sham (sh) group, superior mesenteric artery was exposed but not clamped, In IRI1h, IRI3h, IRI6h and IRI12h groups, the rats were sacrificed at 1, 3, 6 and 12 h after reperfusion, respectively.

Histological examination

After IRI, rats were sacrificed by cervical dislocation and intestine tissue samples were immediately obtained and fixed in 4% formalin and embedded in paraffin, then 5 μm-thick sections were stained with hematoxylin and eosin and examined under light microscope by a blinded pathologist. The Chiu scale [9] of mucosal injury was used to evaluate the degree of histological alteration on 10 sections of 1 mm each to complete 1 cm per animal and then averaged. The scale consists of values from 0 to 5, where 0 normal mucosa; 1, development of sub epithelial (Gruenhagen's) spaces; 2, extension of the sub epithelial space with moderate epithelial lifting from the lamina propria; 3, extensive epithelial lifting with occasional denuded villi tips; 4, denuded villi with exposed lamina propria and dilated capillaries; and 5, disintegration of the lamina propria, hemorrhage, and ulceration.

Electron microscopy

Intestine tissue samples were fixed in lanthanum aldehyde solution (Sigma, St Louis, MO, USA) overnight at 4 °C and then stained by uranium acetate following routine protocols of electron microscopy samples preparation. The stained samples were observed under transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Ussing chamber assay

Transepithelial electrical resistance (TER) of intestine tissues was measured by Ussing chamber assay as described previously [10]. Briefly, Ileal mucosa was stripped and kept in 5 ml Krebs solution oxygenated with 95% O_2—5% CO_2 and circulated in water-jacketed reservoirs at 37 °C. The spontaneous potential difference (PD) was measured using Ringer-agar bridges connected to the electrodes, and the PD was short-circuited to measure short-circuit current ΔIsc. TER ($\Omega\ cm^2$) = PD/ΔIsc.

TUNEL assay

Apoptosis of intestinal epithelial cells was detected by TUNEL assay. Intestine tissue samples were fixed in 4% paraformaldehyde, embedded and cut into 5 μm sections. The sections were then stained with TUNEL kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocols. Each section was observed under a light microscope and the cells in 4 randomly selected fields were counted. Apoptotic index was calculated as: Apoptotic cell number/total cell number.

Real-time PCR

Total RNA was extracted from intestine tissues using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcription was performed using M-MLV Reverse Transcriptase kit

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