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MiR-34c and PlncRNA1 mediated the function of intestinal epithelial barrier by regulating tight junction proteins in inflammatory bowel disease

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ABSTRACT

Background: Inflammatory bowel disease (IBD) is originated from uncontrolled inflammation, and desired methods for IBD therapy remains the main difficult. The network comprised with miRNA and lncRNA has been verified to play an important role on diverse human diseases. In this study, we demonstrated the role of miR-34c and lncRNA PlncRNA1 on the function of intestinal barrier.
Methods: Intestinal epithelial barrier model was constructed based on normal intestinal epithelial cell line Caco-2. 2% DSS was supplemented in the Apical side of the model cells to induce the injury of intestinal epithelial barrier. Real-time PCR or western blot was used to determine mRNA or protein expression of miR-34c, PlncRNA1, Myc-associated zinc finger protein (MAZ), zonula occludens 1 (ZO-1) and occludin.
Results: DSS induced injury of intestinal epithelial barrier, while overexpression of PlncRNA1 seemed to protect intestinal epithelial barrier from injury. Tight junction (TJ) proteins ZO-1 and occludin were regulated by MAZ, while, miR-34c targeted MAZ to regulate its expression, in addition, PlncRNA1 and miR-34c bound together to regulate the expressions of MAZ, ZO-1 and occludin. The protect effects of PlncRNA1 overexpression on intestinal epithelial barrier function was reversed by overexpression of miR-34c.
Conclusion: MAZ and TJ proteins were involved in the function of intestinal epithelial barrier, while miR-34c and PlncRNA1 regulated the intestinal dysfunction cooperatively.
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1. Introduction

Inflammatory bowel disease (IBD) is a class of intestinal diseases with the character of loss intestinal barrier function [1]. However, intestinal mucosal barrier has been verified to play an important role in gastrointestinal microflora. Previous study have suggested that the main role of intestinal mucosal barrier was to prevent the invasion of pathogens [2], while, the intact of intestinal mucosal barrier would bring much invasive bacteria, and break the balance of gastrointestinal microflora. Among this, tight junctions (TJ) is an essential permeable intercellular barrier and regulates the function of intestinal barrier [3]. Several TJ proteins like zonula occludens 1 (ZO-1) and occludin often considered to regulate intestinal permeability. However, the deregulation TJ proteins might also cause unbalance of gastrointestinal microflora. IBD has been identified to originate from this unbalance, and the disease often affects people during their toddler period, and contributes to a reduction of life quality and irreversible negative outcomes such as death [4]. Patients suffering from this disease often develop disabling complications within 5–10 years' diagnosis because of their uncontrolled inflammation [5]. A current evidence suggested that lack of desired methods for IBD therapy was the main difficult for the patients. Therefore, found a promising treatment is necessary.

Recently, increasingly evidences supported that noncoding RNA (ncRNA) potentially regulating most coding genes in human genome, and usually involved in diverse biological functions as well as human diseases. MicroRNAs (miRNAs) are a class of RNA with the

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length of ~22 nt. Presently, miRNAs have been verified to bind to 3'UTR of it's target genes to regulate variety biological processes, such as immune process, cell apoptosis, inflammation reaction, oxidative stress. Unlike miRNA, long noncoding RNAs (lncRNAs) are a set of ncRNA with the length of more than 200 nt, and with less sequence conservation [6]. Now, large amounts of lncRNAs have been well identified and used to regulate gene expression, such as HOTAIR [7,8], MALAT1 [9,10]. Study has supported that both miRNAs and lncRNAs are belong to the ncRNAs, and in most situation, they regulate genes expression and several biological processes cooperatively [11].

There have been reports previously that the well-orchestrated regulatory interaction networks in our body were always involved in diverse ncRNA regulations, for example, miRNA-miRNA interaction, miRNA-mRNA interaction, lncRNA-miRNA interaction [12–14]. Among those, the widespread network lncRNA-miRNA interaction was largely explored, where ncRNAs regulate the expression of RNA by binding the protein coding messengers. For example, lncRNA HULC regulated the onset and progression of hepatocellular carcinoma by binding to miR-372, both of them formed the regulatory network cooperatively [15]. LncRNA MD1 combined with miR-133 and miR-135 to regulate the muscle differentiation [16].

This study we performed lncRNA PlncRNA1 and miR-34c to study whether they regulate intestinal barrier function of IBD cooperatively, and our aims were to explore the potential mechanism underlying the onset and progression of IBD.

2. Materials and methods

2.1. Cell line and culture

The normal intestinal epithelial cell line Caco-2 was purchased from iCell Biotechnology Co., Ltd (China). The cells were cultured in RPBM 1640 medium supplemented with 10% fetal calf serum and maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

2.2. Intestinal epithelial barrier model construction and Trans-epithelial electrical resistance (TER) determination

Caco-2 cells were planted in a Transwell system for monolayers, an epithelial voltohmmeter ERS-2 (Merck Millipore) was used for measurement of TER values. TER was measured until the similar values were occurred on three consecutive measurements. The values were calculated as Ω cm^2. When the TER values of the cells reached at least 500 Ω cm^2 at 20 d, indicating the model was successfully constructed. A total of 2% DSS was added in the Apical side of the model cells to induce the injury of intestinal epithelial barrier.

2.3. Paracellular permeability measurement

After intestinal epithelial barrier model was successfully constructed, the 2% DSS was added in the Apical side of the model and cultured with the supplementary of 1 mg/mL FD-4 solution for 2 h. The paracellular permeability was measured by FD -4 flux. A Synergy H2 microplate reader (Bio Tek) was used for the FD-4 signaling determine.

2.4. MiR-34c and PlncRNA1 transfection

The miR-34c mimic, inhibitor and their negative control (NC) were synthesized from GENEWIZ Biotechnology Co., Ltd (Suzhou, China). Cells were seeded in the 96-well plate for 24 h. Lipofectamine 2000 reagent (Invitrogen, USA) was used for the transfection according to the manufacture's instructions. The transfection efficiency was detected by real-time PCR.

The PlncRNA1 overexpression or down-regulation was constructed based on PlncRNA1 expression plasmid (Invitrogen, USA), then the LV-PincRNA1 or si-Pinc RNA1 was transfected into cells. The empty plasmid served as control.

2.5. Real-time PCR

Total RNA was isolated from cells using an Oligotex Direct mRNA Micro Kit (QIAGEN), then 1 μL RNA was used for quantification. Equal amount of RNA was taken out for the synthesis of cDNA. Real-time PCR was carried out based on the Real-time PCR system (ABI 7900 HT) and PCR Mix(SYBR Green I) according to the manufacture's instructions. The primers used in this study were synthesized from GENEWIZ Biotechnology Co., Ltd (Suzhou, China). GAPDH was considered as internal control.

2.6. Western blot

Proteins were collected after cells were lysed by RIPA buffer (Solarbio) and centrifuged for 20 min. The quality of the proteins was detected by Bradford method. SDS-PAGE was used to separate the proteins with equal amount, the proteins were then transferred onto PVDF membrane, with the primary antibodies of ZO-1 and occludin at 4 °C for 24 h, then the membrane was incubated with the secondary antibodies at room temperature for another 1 h. The antibodies in this study all purchased from Sigma (USA). β-actin acted as internal control.

2.7. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was carried out using Chromatin IP Kit (SOX17 ExactaChip, America) according to the manufacture's instructions. Briefly, cells were fixed and cultured in medium supplemented with glycine in a room temperature. Then the cells were washed and resuspended. The chromatin of the cells was digested by micrococcal nuclease. Immunoprecipitation was carried out by MAZ antibody added into the immunoprecipitation samples, and the samples were cultured at 4 °C in a shaking bed with a gentle shaking. Protein G Agarose Beads was used for collecting immune complex. The chromatin-immune complex was washed by gentle vortex. Finally, DNA crosslinks was reversed and DNA was purified. DNA was amplified by a PCR apparatus. All data obtained from ChIP were repeated for three independent times.

2.8. Luciferase reporter assay

The sequence of MAZ was obtained from the Genebank online. The luciferase reporter plasmid was constructed using the 3'UTR of MAZ. The miR-34c mimic and MAZ-3'UTR vector were co-transfected in the cells according to the manufacture's instruction of Lipofectamine 2000 transfection reagent (Invitrogen). Dual-Luciferase Reporter Assay was used for the luciferase activity measurement.

2.9. RNA-pull down assay

PlncRNA1 and its antisense were transcribed from the specific vector and labeled with Biotin RNA labeling Mix(Roche) and RNA polymerase (Roche) according to the manufacture's instruction. Three independent micrograms of biotin-labeled RNAs were used to construct secondary structure. The cells were then suspended with PBS, and the nuclei was isolated and resuspended by RIP buffer. Folded RNA was mixed with the nuclear extracts of cells in

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