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Septin4 as a novel binding partner of PARP1 contributes to oxidative stress induced human umbilical vein endothelial cells injure

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ABSTRACT

Oxidative stress induced vascular endothelial cell injure is one of the key and initial event in the development of atherosclerosis. Septin4, as a member of GTP binding protein family, is widely expressed in the eukaryotic cells and considered to be an essential component of the cytoskeleton which is involved in many important physiological processes. However, whether Septin4 is involved in cardiovascular diseases, such as oxidative stress inducted endothelial cell injury still unclear. PARP1 as a DNA repair enzyme can be activated by identifying DNA damaged fragments, which consumes high levels of energy and leads to vascular endothelial cell apoptosis. Here, our results first found that Septin4 is involved in oxidative stress induced endothelial cell ROS production and apoptosis through knock-down and over-expression Septin4 approaches. Furthermore, to explore how Septin4 is involved in oxidative stress induced endothelial cells injure, we first identified that Septin4 is a novel PARP1 interacting protein and the interaction is enhanced under oxidative stress. In conclusions, our founding indicates that Septin4 is a novel essential factor involved in oxidative stress induced vascular endothelial cell injury by interacting with apoptosis-related protein PARP1.

1. Introduction

Atherosclerosis is the major risk factor of coronary heart disease, cerebral embolism and peripheral vascular disease, which has greatly threatened human health worldwide. Epidemiology studies showed that the prevalence of atherosclerosis is increasing rapidly in the developing countries, especially in China [1]. Vascular endothelial cells injury is one of the key and initial event in the development of atherosclerosis. Among the factors that induce vascular endothelial cells injure, oxidative stress including accumulation of reactive oxygen species (ROS) is the most common and important factor. Therefore, it is essential to explore the mechanism of oxidative stress induced vascular endothelial cells injury which may provide a new strategy in atherosclerosis treatment.

PARP1 is a DNA repair enzyme which can be activated as a DNA damage receptor by identifying DNA damaged fragments. In addition, PARP1 is also the cutting substrate of caspases which are the core of cell apoptosis [2]. Numerous studies report that oxidative stress activates PARP-1 via DNA damage which consumes high levels of energy and leads to cell apoptosis [3,4]. Therefore, PARP1 plays a key role in DNA damage repair and cell apoptosis. Recent studies considered that PARP1 is an important factor involved in oxidative stress related cardiovascular diseases, including oxidative stress induced endothelial cell injury [5]. Suppression of PARP1 can significantly alleviate cardiovascular diseases both in vivo and in vitro experiments [6,7].

Septin4 is a subtype of Septins family with GTPase activity, which has multiple splicing variants. Septin4 is widely expressed in the eukaryotic cells and mainly located in the chromosome 17q23 [8]. Septin4 is considered to be an essential component of the cytoskeleton and involved in many important physiological processes, such as cell transport and apoptosis [9,10]. Septin4 has been considered as a tumor-inhibiting factor by promoting apoptosis of tumor stem cells [11]. Downregulation expression of Septin4 can significantly decrease the expression of DKK and negatively regulate inflammatory response and liver fibrosis [12]. In addition, Septin4 is involved in the Parkinson's disease by regulating the E3 ubiquitin ligase Nedd4 [13]. However, whether Septin4 is involved in cardiovascular diseases, such as oxidative stress inducted endothelial cell injury still unclear.

Here, we first report that Septin4 is involved in oxidative stress induced human umbilical vein endothelial cells (HUVECs)

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apoptosis and ROS production. Additionally, suppression expression of Septin4 significantly alleviate oxidative stress induced HUVECs apoptosis and ROS production, and over-expression Septin4 has the opposite effect. Moreover, using co-immunoprecipitation assay, we first found that Septin4 interacts with apoptosis-related protein PARP1. Together, our results indicate that Septin4 is involved in oxidative stress induced endothelial cell injury by interacting with PARP1. More broadly, our study provides a potential new regulation pathway of oxidative stress induced endothelial cell injury and indicates that Septin4 suppression may become a new target preventing endothelial cell oxidative stress injury.

2. Materials and methods

2.1. Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were cultured in a humidified incubator at 37 °C with 5% CO_2 and in Dulbecco's modified Eagle medium (DMEM) (HyClone, Logan, UT, USA) with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA), which were obtained from Cambrex (China Center for Type Culture Collection, Wuhan, China). Hydrogen peroxide (H_2O_2) was used to induce HUVECs damage and apoptosis, which was extensively used in vitro models [14–18].

2.2. RNA interference

Control siRNA and Septin4 siRNA were acquired from China RIBOBIO. And Septin4 knockdown were carried out by using jet-prime transfection reagent which were acquired from PolyPlus, France. For Septin4 target gene, we used three effective target sequences to exclude off-target effects. The efficiency of Septin4 knockdown was verified by western blot analysis. The target sequences were as follow:

Septin4 siRNA-1: GGACCGGAAACTTCTTGGT
Septin4 siRNA-2: GGAGATCACTAAGCATGCA
Septin4 siRNA-3: TGGCAGAATACATTGATCA

2.3. Plasmids, antibodies and reagents

Plasmids encoding the full-length human Septin4 (Shanghai Genechem) was cloned to Flag tagged destination vectors according to immunoprecipitation and immunoblotting needs. Antibodies to polyclone rabbit anti-caspase-3 (1:1000; Cell Signaling Technology, USA), monoclonal rabbit anti-PARP1 (1:1000; Cell Signaling Technology, USA), polyclonal rabbit anti-Septin4 (1:1000; proteintech, USA), monoclonal mouse anti-Flag (1:1000; Cell Signaling Technology, USA) and monoclonal mouse anti-β-tubulin (1:2000; abcam, USA) were purchased commercially. Protein A/G immunoprecipitation magnetic beads was obtained from biotool and used for immunoprecipitation.

2.4. Cell transfections and Co-Immunoprecipitation

According to the manufacturer's instructions, plasmid transfections were carried out by Lipofectamine 3000 (Invitrogen, USA). For immunoprecipitation, HUVECs were washed twice and lysed with flag lysis buffer (50 mM Tris, 137 mM NaCl, 1 mM EDTA, 10 mM NaF, 0.1 mM Na3VO4, 1% NP-40, 1 mM DTT, 10% glycerol, pH 7.8, containing fresh protease inhibitors (Roche)). Cell lysates were incubated with anti-PARP1 antibody or anti-Septin4 antibody and 30ul of Protein A/G immunoprecipitation magnetic beads (biotool) for 12 h at 4 °C. And then binding complexes were washed with flag lysis buffer and subjected to SDS-PAGE.

2.5. Cell viability assay

A Cell Counting Kit-8 assay (Dojindo, Kumamoto, Japan) was used to analyze HUVECs viability. In brief, 5×10^3 cells/well were seeded into 96-well plates (NEST Biotechnology) in DMEM supplemented with 10% FBS and were subsequently transfected with control plasmid or Flag-Septin4 plasmid or control siRNA or Septin4 siRNA. HUVECs were subsequently randomly divided into 8 groups: 1: transfected with control plasmid; 2: transfected with control plasmid and exposed to H_2O_2 (150 μmol/l, 250 μmol/l and 500 μmol/l, 12 h); 3: transfected with Flag-Septin4 plasmid; 4: transfected with Flag-Septin4 plasmid and exposed to H_2O_2 (150 μmol/l, 250 μmol/l and 500 μmol/l, 12 h); 5: transfected with control siRNA; 6: transfected with control siRNA and exposed to H_2O_2 (150 μmol/l, 250 μmol/l and 500 μmol/l, 12 h); 7: transfected with Septin4 siRNA; 8: transfected with Septin4 siRNA and exposed to H_2O_2 (150 μmol/l, 250 μmol/l and 500 μmol/l, 12 h). And 100ul CCK-8 solution was added to each well and incubated for 2 h. Cell viability was measured at 450 nm by scanning with a Bio-Rad microplate reader (Model 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.6. Annexin-V and propidium iodide (PI) assay to detect HUVECs apoptosis

Annexin V-fluorescein isothiocyanate (FITC) and PI assay (cat. no. C1062; Beyotime Institute of Biotechnology) were used to detect cell apoptosis, according to the manufacturer's instructions, which was followed by flow-cytometric analysis. 5.0×10^5 cells/well were cultured in 6 well plates, and the various groups were transfected for 36 h. After transfection, cells were exposed to PBS or H_2O_2 (500 μmol/l) for 12 h. And the cells were incubated with 5 μL of Annexin V and 5 μL of PI solution in 500 μl binding buffer for 15 min at room temperature in the dark. A FACSCalibur flow cytometry with FL-1 and FL-2 channel was used to analyze the cells.

2.7. ROS assay

ROS mediated conversion of non-fluorescent 2,7-DCFH-DA to fluorescent DCFH was used to detect endogenous ROS. All groups were transfected for 36 h and exposed to H_2O_2 (500 μmol/l) or PBS for 12 h. The cells were washed with PBS and re-suspended in 500 μl PBS with 2,7-DCFH-DA (5 μM) in the dark for 30 min. The cells were then washed with PBS for three times. And the DCFH was excited at 488 nm and the emission was measured at 525 nm by fluorescence microscopy.

2.8. Western blot assay

After incubating as described above, for the extraction of proteins, cells were lysed in Co-IP buffer and centrifuged at 13000 rpm/min for 20 min at 4 °C. Protein concentrations were determined by the BCA protein assay (Dingguo Changsheng Biotechnology, China) and 40 μg for each sample after blocking with 5% Bovine Serum Albumin (BSA) in Tris Buffered Saline Tween (TBST) at room temperature for 1 h, membranes were incubated overnight at 4 °C with corresponding antibody. Data were normalized to the β-tubulin content of the same sample and determined using Image J software version 1.46 (National Institutes of Health, USA).