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Long non-coding RNA MALAT1 interacts with transcription factor Foxo1 to regulate SIRT1 transcription in high glucose-induced HK-2 cells injury

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ABSTRACT

Background: Tubular injury is considered as a crucial pathological feature of diabetic nephropathy. LncRNA MALAT1 is involved in diabetic complications. Hence the role of MALAT1 in high glucose-induced renal tubular epithelial cells (HK-2) injury deserves investigation.
Methods: The diabetic mice model was established with streptozotocin (STZ) injection. The expression of NEAT1, SIRT1, and Foxo1 mRNA and protein was determined with qRT-PCR and western blot, respectively. The serum creatinine and urinary albumin were examined by enzyme linked immunosorbent assay (ELISA). Interaction between MALAT1 and Foxo1 was detected with RIP and RNA pull-down assay, respectively. Dual luciferase reporter assay was used to evaluate the binding between Foxo1 and SIRT1.
Results: LncRNA MALAT1 was up-regulated in kidney tissues of diabetic mice and in HK-2 cells treated with high glucose, while the expression of SIRT1 was decreased. Interaction between MALAT1 and Foxo1 was observed in HK-2 cells and the interaction was promoted by high glucose treatment. Foxo1 activated SIRT1 transcription by binding to its promoter, and MALAT1 repressed SIRT1 expression through targeting Foxo1.
Conclusion: LncRNA MALAT1 interacts with transcription factor Foxo1 to represses SIRT1 transcription in high glucose incubated HK-2 cells, which promotes high glucose-induced HK-2 cells injury.

1. Introduction

Diabetic nephropathy (DN) refers to the chronic renal impairment caused by diabetes, and it is a leading cause of end-stage renal disease (ESRD) throughout the world [1,2]. As one of the most serious complications of diabetes, diabetic nephropathy has a poor prognosis, speedily developing into renal dysfunction and uremia [3]. However, there are still no effective therapeutic methods in clinic for its complicated pathogenesis. Tubular injury is widely considered as a crucial pathological feature of diabetic nephropathy [4]. Thus, exploration on the mechanism underlying tubular injury under high glucose circumstance may help to seek for efficient targets and prospective therapies for diabetic nephropathy.

Silence information regulator 1 (SIRT1) is a highly conserved NAD^+-dependent deacetylase that expressed in various cells to regulate energy metabolism [5]. SIRT1 has been confirmed to be involved in diabetic nephropathy development, for its role in inhibiting the apoptosis induced by kidney cell injuries, reducing renal inflammation, improving mitochondrial function, and repressing oxidative stress [6]. SIRT1 also has been proclaimed to be vital in the evolution of renal tubulointerstitial fibrosis in diabetic nephropathy [7]. In renal tubular epithelial cells (HK-2), high glucose induced renal tubular epithelial injury by attenuating the deacetylase activity of SIRT1 [8], but the regulatory mechanism still remains uncertain.

As a member of the Forkhead transcription factor of O subfamily, forkhead box protein O1 (Foxo1) activated SIRT1 transcription by binding to SIRT1 promoter region [9]. The activity of Foxo1 has been found to be lowered in the renal cortex of diabetic nephropathy rats, and it inhibited podocyte apoptosis [10]. Besides, Foxo1 protected podocytes from injury induced by high glucose, which partly ameliorated the pathological changes in glomerulus of diabetic nephropathy rats [11], while its influence on tubular injury has not been noted.

The metastasis associated lung adenocarcinoma transcript 1

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(MALAT1) belongs to the long non-coding RNAs (lncRNAs) and it is involved in many diabetic complications. MALAT1 was significantly upregulated in an RF/6 A cell model of hyperglycemia, in the aqueous humor samples, and in fibrovascular membranes of diabetic patients [12]. Knockdown of MALAT1 suppressed retinal endothelial cell proliferation, migration, and tube formation in diabetic rats, thereby alleviating diabetes-induced endothelial cell dysfunction [13]. As well, MALAT1 regulated glucose-induced upregulation of inflammatory mediators IL-6 and TNF-α [14], exhibiting its close correlation with high glucose condition in diabetes. Wu et al. deemed that SIRT1 might be regulated by MALAT1 in liver fibrosis [15]. With bioinformatics analysis, we further discovered the binding site between MALAT1 and Foxo1, hinting potential interaction between them. Therefore, we deduced that MALAT1 may negatively regulate SIRT1 through targeting Foxo1, to contribute the high glucose-induced renal tubular epithelial injury. This study was undertaken to illuminate the role of MALAT1 in glucose-induced renal tubular epithelial injury, aiming to uncover the molecular mechanism of tubular injury in diabetic nephropathy.

2. Materials and methods

2.1. Animal models establishment and drug treatment

Streptozotocin (STZ) was purchased from Sigma (USA) and dissolved in the citrate buffer (0.1 M, pH 4.5) for filtration sterilization. Male C57BL/6 mice (aged 7 weeks, n = 10/group) were randomized to receive a daily intraperitoneal injection of either 50 mg/kg STZ in citrate buffer or 0.1 M citrate buffer (pH 4.5) for 5 consecutive days according to the protocol recommended by the Animal Models of Diabetes Complications Consortium (AMDCC) [16]. All the Animals were kept on a standard diet (0.2% sodium) and water ad libitum. Two weeks after the initial intraperitoneal injection, the blood glucose was detected from the tail artery by a non-invasive automated sphygmomanometer (IITC, USA) and animals with a blood glucose >16 mmol/L were considered diabetic. Mice were kept in individual metabolic cages for 24 h urine collection at the end of 10 weeks' treatment. Mice were then anesthetized with pentobarbital sodium and the kidneys were harvested immediately, and the kidney tissues were snap-frozen in liquid nitrogen and stored at −80 °C for total RNA and protein extraction. Our study on animals was approved by the Animal Care Committee of the First Affiliated Hospital of Soochow University. All protocols of animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals proposed by the Chinese National Institutes of Health.

2.2. Estimation of serum creatinine and urinary albumin

The enzyme linked immunosorbent assay (ELISA) was used for estimation of serum creatinine and urinary albumin by using the related ELISA kits (BD Bioscience, USA). Assay diluent and standard or samples were added to each well and incubated for 2 h at room temperature, and the plates were washed four times to remove any unbound substances. Then conjugate (a polyclonal antibody conjugated to horseradish peroxidase) was added to each well for 1 h incubation at room temperature, and the plates were washed four times before substrate solution was added to each well. Color development was performed with a 20min-incubation at room temperature, and then stop solution was added to each well to measure the intensity of the coloring. The absorbance at 450 nm was detected using an ELISA reader. The standard curve was created using the standard preparation supplied with the assay. Each sample was measured for triplicate.

2.3. Quantitative real-time PCR (qRT-PCR)

TRIzol reagent (Invitrogen) was used to isolate the total RNA from kidney tissue of mice. The extracted RNA was reversely transcribed to cDNA with an iScript kit (Bio-Rad, USA). Then qRT-PCR was conducted with SYBR® Premix DimerEraser kit (TaKaRa) on an ABI PRISM 7300 R T-PCR system (Applied Biosystems, USA). $2^{-\Delta\Delta Ct}$ method was used to quantify the related level of MALAT1 and SIRT1 mRNA expression. The specific primers used were synthesized by Sangon (Shang, China).

2.4. Western blot assay

The protein levels of SIRT1 and Foxo1 was assessed via western blot assay. Total protein was extracted from kidney tissue of mice, and quantified with a BCA protein assay kit (Beyotime, China). All samples were separated with SDS-PAGE, followed by transferred onto the polyvinylidene difluoride (PVDF) membrane (Millipore, USA). After blocked with 5% powdered milk in Tris-buffer containing 0.05% Tween 20 at room temperature for 1 h, the membranes were probed with the primary antibody against SIRT1, Foxo1, and beta-actin (Abcam, UK) at 4 °C for overnight, followed by maintained with horseradish peroxidase-labeled secondary antibody (Abcam, UK) at room temperature for 2 h after washed three times. The enhanced chemiluminescent (ECL) detection systems were used to visualize the protein bands.

2.5. HK-2 cells culture and cell transfection

HK-2 cells were commercially obtained from American Type Culture Collection (ATCC, USA) and maintained in Dubecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin (100U/ml)-Streptomycin (100 μg/ml). HK-2 cells were cultured at 37 °C with 5% CO_2 until 60–70% confluence. Then cells incubated in serum-free culture were treated with normal glucose (NG, 5.5 mM) or high glucose (HG, 45 mM) to investigate the mechanism underlying high glucose induced cell injury. All cells were incubated with glucose for 48 h.

For cell transfection, HK-2 cells were seeded in 24-well plates (1×10^4/well) and transfected with small interfering RNA targeting MALAT1 (si-MALAT1) (Ribobio, Guangzhou, China) with a final concentration of 50–100 nM. Cell transfection was performed using Lipofectamine 2000 and cells were kept under condition of 5% CO_2 at 37 °C for 48 h. After incubation, fresh medium was used and cells were treated with high glucose (45 mM) for another 48 h (HG + si-MALAT1).

2.6. MTT assay

After HK-2 cells stimulated with high glucose, the cell viability was assessed using MTT assay with a MTT cell proliferation assay kit (Cayman). HK-2 cells were diluted to 1×10^4 cells/ml with serum-free DMEM and seeded in 96-well plates (100μl/well). After 20 μl of methylthiazoletetrazolium (MTT; 5 mg/ml) added to each well, the plates were incubated for another 4 h at 37 °C in humidified 5% CO_2 in air. The supernatant was then discarded and 150 μl of dimethylsulfoxide (DMSO) was added to each well to dissolve blue formazan crystals. The absorbance was measured with a microplate reader (Bio-Rad, USA) at a wavelength of 490 nm. The cell viability was calculated with the cells in normal glucose group served as a control.

2.7. RNA immunoprecipitation (RIP)

RIP assay was performed to estimate the binding between

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