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LncRNA Uc.173 is a key molecule for the regulation of lead-induced renal tubular epithelial cell apoptosis

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ABSTRACT

Transcribed ultra-conserved region (T-UCR) transcripts are a novel class of long non-coding RNAs (lncRNAs) transcribed from ultra-conserved region which is highly conserved in human, rat, and mouse genome. LncRNA UC.173 has been found significantly down-regulated in lead-exposed population and lead-exposed animal mode, and had an inhibitory effect on lead-induced nerve cell apoptosis. We supposed that lncRNA UC.173 had an inhibitory effect on lead-induced renal tubular epithelial cell apoptosis. Thus, the aim of our study was to explore the function of lncRNA UC.173 in lead-exposed renal tubular epithelial cells. In our results, lead exposure inhibited renal tubular epithelial cells viability and promoted cell apoptosis and apoptosis-associated genes expression, but no effect on cell-cycle distribution. Lead exposure inhibited the expression of lncRNA UC.173 in renal tubular epithelial cells, and the inhibition effect was time-dependent and concentration-dependent. Up-regulation of lncRNA UC.173 had no effect on renal tubular epithelial cell viability, cell cycle and apoptosis, but significantly rescued lead-induced inhibition of renal tubular epithelial cell viability and suppressed lead-induced cell apoptosis. In summary, our experiments suggest that lncRNA UC.173 is certainly involved in the regulation of lead-induced renal tubular epithelial cell apoptosis, which may supply a new strategy to minimize lead-induced nephrotoxicity.

1. Introduction

Lead (Pb) is one of the main heavy metals and widely distributed in the environment [1]. Lead exposure can cause pathological changes to multiple organ systems, including haemopoietic system [2], digestive system [3], immune system [4], nervous system [5], reproductive system [6], urinary system [7], circulatory system [8], respiratory system [9], endocrine system [10], and kinetic system [11]. Kidney is one of the most sensitive target organs for lead toxicity, while the proximal tubule is the major site of lead-induced renal injury [12–14]. Up to now, the mechanism of lead-induced renal injury is still unclear.

Long non-coding RNAs (lncRNAs) are defined as non-coding RNAs longer than 200 nucleotides [15]. Transcribed ultra-conserved region (T-UCR) transcripts are a novel class of lncRNAs transcribed from ultra-conserved regions (UCRs). T-UCRs are absolutely conserved between the orthologous regions of the human, rat, and mouse genomes [16]. Although the biological functions of T-UCRs are still unclear, more and more studies are suggesting T-UCRs involve in various pathological and physiological processes [17].

LncRNA UC.173 is a lncRNA from a transcribed ultra-conservative region. The expression of lncRNA UC.173 has been found decreased in serum of lead-exposed children and lead-exposed animal model [18]. Meanwhile, lncRNA UC.173 inhibited lead-induced apoptosis in nerve cells [18]. Thus, we supposed that lncRNA UC.173 had an inhibitory effect on lead-induced renal tubular epithelial cell apoptosis. In order to confirm this guess, we explored the biological functions of lead and lncRNA UC.173 in renal tubular epithelial cells and the association between lead and lncRNA UC.173 in renal tubular epithelial cell apoptosis.

2. Materials and methods

2.1. Cell lines

Human proximal tubular epithelial cell line (HK-2) and human kidney proximal tubular epithelial cell line (HKC) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. HK-2 cells were cultured in Dulbecco's modified Eagle's

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medium/F12 (Invitrogen, USA), supplemented with 10% fetal calf serum. HKC cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, USA) supplemented with 10% fetal calf serum. All cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Construction of lead-exposed cell model and cell transfection

The lead-exposed cell model was constructed by treating cells with 0.1 μmol/L lead acetate solution (PbAc). The coding sequence region of human lncRNA UC.173 was respectively amplified from cDNA and cloned into pcDNA3.1 express vector, which were used to up-regulate lncRNA UC.173 expression in vitro. The plasmid was synthesized from GeneChem Inc. (China) and the resulting constructs were confirmed by DNA sequencing. Cells were transfected using lipofectamine 3000 reagent (Invitrogen, USA) in Opti-MEM (Gibco, USA), according to the manufacturer's instructions.

2.3. CCK-8

The cell viability in vitro was assessed by using CCK-8 assay. Briefly, 1×10^3 cells were seeded into a 96-well plate with sextuple repeat for each condition. The cells were incubated for 12 h, 24 h or 48 h with 1.0 μm/L lead acetate solution and/or plasmid, Ten microliters of CCK8 (Cell Counting Kit-8, Beyotime, China) was added to each well and incubated for 2 h. The absorbance value (OD) of each well was measured at 450 nm. For each experimental condition, 6 wells were used. Experiments were performed three times.

2.4. Cell cycle analysis

Cells exposed with lead or/and transfected with pcDNA3.1-lncRNA UC.173 were harvested after 48 h. rinsed with cold PBS and fixed with 70% ice-cold ethanol for 48 h at 4 °C. Fixed cells were rinsed with cold PBS followed by incubation with PBS containing 10 mg/ml propidium iodide and 0.5 mg/ml RNase A for 30 min at 37 °C. The DNA content of labeled cells was acquired using FACS cytometry assay (BD Biosciences, USA). The percentage of the cells in G0-G1, S, and G2-M phases were counted and compared.

2.5. Apoptosis assay

The apoptosis ratio was analyzed using the Annexin V-FITC Apoptosis Detection Kit (Beyotime, China). At 48 h after lead-exposure and/or transfection, cells were harvested and resuspended in binding buffer containing Annexin V-FITC and PI according to the manufacturer's instructions. The samples were analyzed by flow cytometry (BD Biosciences, USA). Cells were discriminated into viable cells, necrotic cells, and apoptotic cells by using BD FACSDiva 6.1.3 software (BD Biosciences, USA), and then the percentages of apoptotic cells from each group were compared. Experiments were performed three times.

2.6. Quantitative real time PCR

Total RNA was extracted from tissues cell lines using RNAiso Plus (Takara, Japan), and then reverse-transcribed to cDNAs using the PrimeScript RT Master Mix (Takara, Japan), according to manufacturer instructions. The LightCycler (Roche, USA) was selected to conduct the amplification of cDNAs using SYBR Premix Ex TaqTM II (Takara, Japan). The primers for RT-PCR were purchased from Takara. Relative expression was calculated via the comparative cycle threshold method and was normalized to the expression of GAPDH. Tests were repeated in triplicate.

2.7. Western blot

Cells were lysed in RIPA buffer (Cwbiotech, China), and protein concentrations were measured using the BCA protein assay kit (Beyotime, China). Then proteins in samples were separated through SDS-PAGE, and subsequently transferred to a PVDF membrane (Millipore, USA). After the membrane was blocked for 2h using 5% skim milk, it was incubated with primary antibodies against Caspase-3, Caspase-9, Cleaved Caspase-3, Cleaved Caspase-9 (Cell Signaling Technology, USA), Fas, FasL, Bax, Bcl-2 (Abcam, USA) and β-actin (Cwbiotech, China) for 2 h. Finally, the membrane was incubated with HPR-conjugated secondary antibodies (Cell Signaling Technology, USA) for 2 h. Signals were detected using enhanced chemiluminescence reagents (Pierce, USA). Quantity One Software (Bio-Rad) was used to analyze the intensity of blots.

2.8. Statistical analysis

Statistical analyses were accomplished using SPSS 17.0 and diagrams were conducted using GraphPad prism 5.0. The Student's t test was used for comparisons of two independent groups. One-way ANOVA was used to determine the differences between groups. The statistically significant difference was set at $P < .05$.

3. Results

3.1. Lead exposure inhibits renal tubular epithelial cell viability and promotes cell apoptosis

To explore the effect of lead on cell viability, HK-2 and HKC cells were exposed with lead acetate solution. As shown in Fig. 1A, the results of CCK-8 assay displayed that lead exposure significantly decreased HK-2 and HKC cells viability in comparison to control cells after exposed 24 h and 48 h ($P < .05$). The cell cycle analysis showed lead exposure had no effect on cell-cycle distribution in HK-2 and HKC cells (Fig. 1B, $P > .05$). The apoptosis assay suggested that the percentage of apoptotic cells in lead exposure HK-2 and HKC cells were higher than their control cells (Fig. 1C, $P < .05$). We further detected apoptosis-associated genes expression through western blot, and found lead exposure significantly increased the expression of Caspase-3, Caspase-9, Cleaved Caspase-3 and Cleaved Caspase-9 in HK-2 and HKC cells (Fig. 1D). However, lead exposure has no effect on the expression of Fas, FasL, Bax and Bcl-2 (Fig. S1).

3.2. The association between lead exposure and lncRNA UC.173 in renal tubular epithelial cells

To study the association between lead exposure and lncRNA UC.173 in renal tubular epithelial cells, we treated HK-2 and HKC cells with lead acetate solution and observed the changes of lncRNA UC.173 expression at various lead concentrations and various treatment time points. We detected the expression of lncRNA UC.173 in HK-2 and HKC cells at four lead concentrations (0 μmol/L, 0.1 μmol/L, 0.5 μmol/L and 1.0 μmol/L), and found the expression of lncRNA UC.173 was gradually decreased along with the raise of lead concentrations (Fig. 2A). We determined the expression of lncRNA UC.173 in HK-2 and HKC cells at four time points (0 h, 12 h, 24 h and 48 h) after the treatment, and found the expression of lncRNA UC.173 was gradually decreased along with the extension of lead-exposure time (Fig. 2B). There results suggested lead exposure inhibited the expression of lncRNA UC.173 in renal tubular epithelial cells, and the inhibition effect was time-dependent and concentration-dependent.

3.3. Overexpression of lncRNA UC.173 has no effect on renal tubular epithelial cells viability, cell cycle and apoptosis

To explore the effect of lncRNA UC.173 on renal tubular epithelial cells, we induced up-regulation of lncRNA UC.173 by transfecting lncRNA UC.173 plasmid, and these efficiencies were confirmed by qRT-