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Omentin-1 protects renal function of mice with type 2 diabetic nephropathy via regulating miR-27a-Nrf2/Keap1 axis

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ABSTRACT

Omentin-1, a novel identified adipokine, always significantly decreases in patients with metabolic syndrome. However, the functional roles of omentin-1 in diabetic nephropathy (DN) remains largely unknown. In the present study, we found that omentin-1 treatment could improve renal function of type 2 diabetic db/db mice. ELISA assay and immunohistochemistry staining showed that omentin-1 reduced the productions of proinflammatory cytokines (IFN-γ, TNF-α, MCP-1 and IL-8), and improved oxidative stress level (CAT, MDA and SOD) in the kidney tissue, indicating omentin-1 could relieved the inflammatory response and suppressed oxidative stress. Mechanistic analysis demonstrated that omentin-1 down-regulated miR-27a expression, and subsequently inhibited oxidative stress and inflammation. Luciferase reporter assay and western blot further revealed that miR-27a directly targeted the 3′ untranslated region (UTR) of nuclear factor erythroid 2-like 2 (Nrf2) and reduced its expression in type 2 DN. Taken together, these findings provide a new function of omentin-1 in renal protection and also delineate multiple potential targets for therapeutic intervention for type 2 DN.

1. Introduction

Diabetic nephropathy (DN) is the major cause of end-stage renal failure and cardiovascular disease worldwide [1,2]. Accumulating evidences suggest that DN is a multifunctional degenerative disorder associated with hyperglycemia-induced oxidative stress and inflammation [3,4]. Some studies have demonstrated that hyperglycemia increases the free radicals production and induces oxidative stress which contributes to the pathogenesis and progression of DN [5]. Thus, it might be a smart strategy to identify and develop novel therapeutic alternatives targeting oxidative stress-inflammatory cytokine signaling to prevent the rapid prevalence of DN.

Omentin-1 is a novel identified adipokine preferentially produced by visceral stromal vascular cells [6,7]. Previous studies demonstrated that circulating omentin-1 levels are significantly decreased in patients with obesity, insulin resistance, diabetes, metabolic syndrome and cardiovascular disease [8–11]. In atherogenesis, omentin-1 not only suppresses inflammatory response and foam cell formation in macrophages, but also inhibits the migration, proliferation, and collagen production of vascular smooth muscle cells [12]. In osteoarthritis, Li et al found that omentin-1 exerts a chondro-protective effect and suppresses matrix metalloproteinases expression by inactivating JAK-2/STAT3 pathway [13]. In hepatocellular carcinoma, omentin-1 promotes cell apoptosis through inhibiting silent mating type information regulation 2 homolog 1 (Sirt1)-dependent p53 deacetylation [14]. Taken together, these studies support a protective role of omentin-1 in many diseases and may be a potential therapeutic target for DN. However, the role of omentin-1 in DN still remains largely unknown.

MicroRNAs (miRNAs) are endogenous non-coding RNAs that negatively regulate gene expression by binding to the 3′ untranslated regions (UTRs) of target mRNAs [15,16]. miRNAs could regulate specific target genes involved in many physiological and pathological processes in various diseases, including DN [17,18].

Using type 2 diabetic db/db mice model, the study was aimed to explore the effect of omentin-1 on renal function of type 2 DN and we found that omentin-1 could protect renal function by suppressing inflammatory response and oxidative stress. Further studies revealed that omentin-1 could down-regulate miR-27a expression and subsequently inhibit oxidative stress by targeting Nrf2/Keap1 pathway.

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2. Material and methods

2.1. Cell culture and transfection

NRK-52E, HK-2 and HBZY-1 cell lines were purchased from China Center for Type Culture Collection (Wuhan, China). Cells were cultured in DMEM (30 mmol/L D-glucose) supplemented with 10% FBS at 37 °C in a humidified atmosphere with 5% CO2. Human miR-27a mimics and miRNA negative control were purchased from GenePharma (Shanghai, China). The full-length human Nrf2 was amplified by PCR and subsequently cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) at BamHI and XhoI site. Cells were transfected with 50 nM miR-27a mimics and miRNA mimics negative control, 50 nM pcDNA3.1-Nrf2 and pcDNA3.1 (Promega, Madison, WI) using lipofectamine 2000 (Life Technologies).

2.2. Mice

The 8-week-old male db/db diabetic mice and their nondiabetic littermate control db/m mice weighing 18–22 g in the C57BLKS/JNju background were purchased from the Comparative Medicine Center of Yangzhou University. All mice were housed separately and maintained on a 12-h light/dark cycle, temperature 23 ± 3 ℃, humidity 55 ± 15% with free access to water and food. The db/m mice (n = 20) were randomly divided into two groups: normal control (NC) group and normal Ad-Omentin-1 treatment (N-Ome) group; db/db mice (n = 20) were randomly divided into two groups: model control (MC) group and model Ad-Omentin-1 treatment (M-Ome) group.

Adenovirus producing the full-length human omentin-1 was prepared using the Adenovirus Expression Vector Kit (Takara, Kyoto, Japan). NC and MC mice were administered via tail vein injection with Ad-Gal at a dose of 1×10^8 plaque-forming units/kg once every 2 weeks for a total of 8 weeks. N-Ome and M-Ome mice were administered via tail vein injection with Ad-omentin-1 at a dose of 1×10^8 plaque-forming units/kg once every 2 weeks for a total of 8 weeks. Two weeks after the final administration, the mice were sacrificed. All animal experimental procedures were performed in accordance with guidelines of the Animal Ethical and Experimental Committee of Putuo Hospital affiliated to Shanghai University of Traditional Chinese Medicine.

2.3. Measurement of cytokines in serum

The concentrations of IFN-γ (#H025), TNF-α (#H052), MCP-1 (#H115) and IL-17 (#H014) in the mouse sera were measured with ELISA kits (Nanjing Jiancheng Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions.

2.4. Histopathological examinations

After weighing, part of kidney in each group was frozen in liquid nitrogen and stored at -80 ℃ for biochemical assays, and the rest of kidney were fixed in 10% neutral-buffered formalin, embedded by paraffin, and stained with hematoxylin and eosin (HE).

2.5. Immunohistochemistry staining

Formalin-fixed, paraffin-embedded renal tissue sections (4 μm) were prepared, and the levels of catalase (CAT, #ab16731), malondialdehyde (MDA, #ab6463) and superoxide dismutase (SOD, #ab13498) (1:150; Abcam, Cambridge, MA, USA) were analyzed using immunohistochemistry staining following the standard protocol. The mean density was analyzed with Image Pro plus 6.0 software.

2.6. Enzyme activity assay

Part of kidney in each group was homogenized with a blade-blender homogenizer and then centrifuged for 20 min at the speed of 3000 rpm and the supernatant was collected. The levels of SOD (#A001-3), CAT (#A007-2) and MDA (#A003-1) were determined using ELISA kits (Nanjing Jiancheng Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions.

2.7. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen). The expression of miR-27a was evaluated using Taqman miRNA Assays (Applied Biosystems, Foster City, CA). The mRNA levels of Nrf2 and HO-1 were determined using SYBR Green PCR master mix (Applied Biosystems). U6 snRNA or β-actin was used as an endogenous control. All samples were normalized to internal controls and fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method.

The primer sequences are as follows:
Nrf2 Forward: 5′-ATGCCCTCACCTGCTACTTT-3′,
Reverse: 5′-AGGCCAAGTAGTGTGTCTCC-3′;
HO-1 Forward: 5′-ATTCTCTTGGCTGGCTTCCT-3′,
Reverse: 5′−CCCCTCTGAAGTTTAGGCCA-3′,
β-actin Forward: 5′-GGCATCCTCACCCTGAAGTA-3′,
Reverse: 5′-TAGCACAGCCTGGATAGCAA-3′,

2.8. Luciferase reporter assay

A fragment of the 3′UTR of Nrf2 containing the putative target sites of miR-27a was designed and synthesized and then cloned into the pGL3 control vector (Promega, Madison, WI) downstream of the firefly luciferase gene. For luciferase reporter assays, HBZY-1 cells were co-transfected with pGL3-Nrf2 or pGL3-control vector, together with miR-27a mimics or the negative control. After 48 h, Renilla luciferase activity was measured using the dual luciferase reporter assay system (Promega).

2.9. Western blot

Part of renal tissues in each group was homogenized with RIPA lysis buffer for immunoblot analysis. Proteins were separated using 10% SDS-PAGE gels, and transferred to PVDF (polyvinylidene fluoride) membranes (Millipore, Billerica, MA, USA). Then, the membranes were incubated with primary antibodies purchased from Abcam as follows: monoclonal rabbit anti-Nrf2 (#ab62352) and polyclonal rabbit anti-1 (#ab13243), and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Bands were detected with electro-generated chemiluminescence (ECL), and the protein expression levels were normalized to β-actin (monoclonal rabbit antibody, #ab32575, Abcam).

2.10. Statistical analysis

Data were expressed as means ± SD from at least three independent experiments. Data were analyzed by the 2-tailed Student's *t*-test or one-way ANOVA with Scheffe's post-hoc analysis as appropriate. Values with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Omentin-1 protects renal function of type 2 DN mice

To investigate the effect of omentin-1 on renal function of type 2 DN mice (Fig. 1A), we first determined the kidney weight and body weight of each group. After eight weeks treatment with omentin-1, both kidney and body weight of M-Ome were significantly lower than MC group, but