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Research Paper

Polydatin prevents fructose-induced liver inflammation and lipid deposition through increasing miR-200a to regulate Keap1/Nrf2 pathway

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ABSTRACT

Oxidative stress is a critical factor in nonalcoholic fatty liver disease pathogenesis. MicroRNA-200a (miR-200a) is reported to target Kelch-like ECH-associated protein 1 (Keap1), which regulates nuclear factor erythroid 2-related factor 2 (Nrf2) anti-oxidant pathway. Polydatin (3,4′,5-trihydroxy-stilbene-3-β-D-glucoside), a polyphenol found in the rhizome of *Polygonum cuspidatum*, have anti-oxidative, anti-inflammatory and anti-hyperlipidemic effects. However, whether miR-200a controls Keap1/Nrf2 pathway in fructose-induced liver inflammation and lipid deposition and the blockade of polydatin are still not clear. Here, we detected miR-200a down-regulation, Keap1 up-regulation, Nrf2 antioxidant pathway inactivation, ROS-driven thioredoxin-interacting protein (TXNIP) over-expression, NOD-like receptor (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome activation and dysregulation of peroxisome proliferator activated receptor-α (PPAR-α), carnitine palmitoyl transferase-1 (CPT-1), sterol regulatory element binging protein 1 (SREBP-1) and stearoyl-CoA desaturase-1 (SCD-1) in rat livers, BRL-3A and HepG2 cells under high fructose induction. Furthermore, the data from the treatment or transfection of miR-200a minic, *Keap1* and *TXNIP* siRNA, Nrf2 activator and ROS inhibitor demonstrated that fructose-induced miR-200a low-expression increased Keap1 to block Nrf2 antioxidant pathway, and then enhanced ROS-driven TXNIP to activate NLRP3 inflammasome and disturb lipid metabolism-related proteins, causing inflammation and lipid deposition in BRL-3A cells. We also found that polydatin up-regulated miR-200a to inhibit Keap1 and activate Nrf2 antioxidant pathway, resulting in attenuation of these disturbances in these animal and cell models. These findings provide a novel pathological mechanism of fructose-induced redox status imbalance and suggest that the enhancement of miR-200a to control Keap1/Nrf2 pathway by polydatin is a therapeutic strategy for fructose-associated liver inflammation and lipid deposition.

1. Introduction

Clinical and experimental evidences demonstrate that excessive fructose consumption causes nonalcoholic fatty liver disease pathogenesis [1,2]. Oxidative stress is considered to be a critical factor in this pathogenesis [3]. However, the mechanisms of fructose-induced redox status imbalance in liver inflammation and lipid accumulation are still poorly elucidated. Kelch-like ECH-associated protein 1 (Keap1) directly leads to continual ubiquitination and subsequent degradation of the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in cell cytoplasm [4]. Upon dissociated from Keap1, Nrf2 is translocated into nuclear, and then initiates phase II detoxification and antioxidant defense enzyme to counteract oxidative stress and modulate redox status balance [5]. Keap1 ablation increases Nrf2 nuclear translocation and up-regulates hemeoxygenase-1 (HO-1), attenuates palmitate-induced reactive oxygen species (ROS) production in human hepatoblastoma cell line (HepG2) [6]. Nrf2 knockout develops thioredoxin-interacting protein (TXNIP) and nucleotide-binding domain (NOD)-like

Abbreviations: ASC, apoptosis-associated speck-like protein; CPT-1, carnitine palmitoyl transferase-1; GST, glutathione S-transferase; HO-1, hemeoxygenase-1; H2O2, hydrogen peroxide; IL, interleukin; Keap1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; miR-200a, microRNA-200a; NAC, N-acetyl-L-cysteine; NAFLD, non-alcoholic fatty liver disease; NLRP3, the NOD-like receptor (NLR) family, pyrin domain containing 3; NQO1, NAD(P)H, quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; PPAR-α, peroxisome proliferator activated receptor-α; ROS, reactive oxygen species; SCD-1, stearoyl-CoA desaturase-1; SREBP-1, sterol regulatory element binging protein 1; tBHQ, *tert*-butylhydroquinone; TNF-α, tumor necrosis factor-α; TXNIP, thioredoxin-interacting protein

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receptor protein 3 (NLRP3) high-expression in lipopolysaccharide (LPS)-induced lung inflammation of mice [7]. TXNIP can activate nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3) inflammasome and subsequently generate interlin-1β (IL-1β) in response to oxidative stress [8]. Nrf2 activation simulated by *tert*-butylhydroquinone (tBHQ) increases NAD(P)H: quinone oxidoreductase 1 (NQO1) to scavenge ROS, this process inhibits NLRP3 inflammasome activation and IL-1β production in adenosine triphosphate and lipopolysaccharide-exposed human THP-1 cell- and bone marrow-derived macrophages [9]. Thus, anti-oxidation function of the Nrf2 pathway may contribute to the elimination of ROS and subsequent inflammatory response [10]. Specific Keap1 knockout blocks ethanol-inducted increase of liver triglyceride (TG) levels and up-regulation of sterol regulatory element-binding protein 1 (SREBP-1) and its target gene stearoyl-CoA desaturase 1 (SCD-1) in Nrf2-null mice [11]. Our previous study showed that ROS-induced TXNIP drove fructose-mediated hepatic inflammation and lipid deposition through NLRP3 inflammasome activation [2]. However, it remains unclear whether and how Keap1/Nrf2 pathway interferes with this event.

There is crosstalk between oxidative stress and microRNA-200a (miR-200a). MiR-200a high-expression by targeting Keap1 inhibition up-regulates Nrf2 nuclear translocation as well as glutathione S-transferase (GST), hemeoxygenase-1 (HO-1) and NQO1 protein levels, and then decreases ROS production in human adult cardiomyocyte line exposed to hypoxia [12], and increases tumor necrosis factor-α (TNF-α) and IL-6 at mRNA levels, as well as down-regulates peroxisome proliferator activated receptor-α (PPAR-α), and up-regulates SCD1 and SREBP-1c at protein levels in fructose-exposed HepG2 cells [13]. It is attractive therefore to hypothesize that aberrant miR-200a expression may target to Keap1 and then inhibit Nrf2 antioxidant pathway, participating in fructose-induced liver inflammation and lipid deposition.

Anti-oxidant agent polydatin (3,4′,5-trihydroxy-stilbene-3-β-D-glucoside), a natural precursor of resveratrol, is isolated from *Polygonum cuspidatum* Siebold & Zucc, which has been used in traditional Chinese medicine to treat liver disorders associated with oxidative stress, inflammation and lipid deposition for centuries in patients [14] and in experimental animals [15,16]. Polydatin decreases malondialdehyde (MDA) levels and increases GST activity in the liver of carbon tetrachloride- or D-galactose-stimulated mice, with the reduction of TNF-α and IL-1β gene expression [17,18]. It inhibits Keap1, promotes Nrf2 transcriptional activity, increases HO-1 protein levels, and quenches ROS generation in advanced glycation-end products-simulated rat glomerular mesangial cells [19]. Additionally, polydatin suppresses kidney NLRP3 inflammasome activation in potassium oxonate-treated rats [20], increases liver PPAR-α protein levels in streptozocin-induced diabetic mice fed with high-fat and sugar diet [21], and decreases liver SCD-1 protein levels in high-fat diet-fed rats [22]. Thus, it is important to understand the molecular mechanism underlying its attenuation of fructose-induced redox status imbalance in liver inflammation and lipid deposition.

In this study, we showed that high fructose decreased miR-200a to target Keap1 and inhibit Nrf2 antioxidant pathway, and then triggered TXNIP-activated NLRP3 inflammasome, causing liver inflammation and lipid deposition. Furthermore, polydatin reduced oxidative stress by increasing miR-200a to regulate Keap1/Nrf2 pathway, resulting in the protection against fructose-induced liver inflammation and lipid deposition. Therefore, the ability of high miR-200a expression to control Keap1/Nrf2 pathway by polydatin may be a new therapeutic strategy.

2. Materials and methods

Reagents For animal experiments, fructose was provided from Shandong Xiwang Sager Industry Co., Ltd. (Binzhou, China), polydatin (purity ≥ 98%) was obtained from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China), and pioglitazone table was purchased from Jiangsu DeYuan Pharmaceutical Co., Ltd. (Lianyungang, China). For cell experiments, fructose, polydatin, pioglitazone, tBHQ, *N*-acetyl-L-cysteine (NAC) and 2′ 7′-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Kits of TG, total cholesterol (TC), MDA, hydrogen peroxide (H_2O_2) as well as enzyme-linked immunosorbent assay (ELISA) kits of IL-1β and TNF-α were purchased from Jiancheng Biotechnology Co., Ltd (Nanjing, China), respectively. Kits of nuclear protein and cytoplasmic protein extraction were obtained from Keygen Biotechnology Corp., Ltd (Nanjing, China). Fetal bovine serum was purchased from Wisent Technology (St-Bruno, QC, Canada). DMEM and opti-MEM were purchased from Basal Media Biotechnology Co., Ltd (Shanghai, China). Lipofectamine 2000 was got from Invitrogen Corporation (Carlsbad, CA, USA). Dual-luciferase reporter assay system kit was purchased from Promega Corporation (Madison, USA). Trizol reagent was got from Sigma-Aldrich Inc. (St. Louis, MO). Reverse Transcription System Kit, dNTPs and RNase inhibitor were obtained from Vazyme Biotechnology Co., Ltd (Nanjing, China), respectively. iTaqTM Universal SYBR® Green Supermix was got from Bio-Rad Inc. (California, USA). MultiScribe reverse transcriptase was obtained from Promega Corporation (Wisconsin, USA). Cell lysis buffer was purchased from Beyotime Biotechnology (Nanjing, China, P0013). Pierce™ BCA protein assay kit was purchased from Thermo Scientific (Schwerte, Germany). Rabbit anti-Nrf2 (sc-722), mouse anti-HO-1 (sc-136960), mouse anti-TXNIP (sc-2712138), rabbit anti-ASC (sc-22514), rabbit anti-Caspase-1 (sc-514), rabbit anti-pro-Caspase-1 (sc-514), rabbit anti-PPAR-α (sc-9000), rabbit anti-CPT-1 (sc-139480), rabbit anti-SREBP-1 (sc-367), rabbit anti-SCD-1 (sc-14720) and rabbit anti-GAPDH (sc-25778) were purchased from Santa Cruz Biotechnology Co., Ltd (Santa Cruz, CA, USA). Rabbit anti-Keap1 (#8047), mouse anti-Lamin A/C (#4777), rabbit anti-GST (#2625), mouse anti-NQO1 (#3187), rabbit anti-NLRP3 (#13158) and HRP-conjugated rabbit anti-IgG (#AP132P) were obtained from Cell Signaling Technology (Cambridge, USA). Mouse anti-IL-1β (MAB5011), mouse anti-pro-IL-1β (MAB5011) and HRP-conjugated mouse anti-IgG (HAF007) were got from R&D System (Minneapolis, USA). Mouse anti-β-actin (ABM-0001) was purchased from Zoonbio Biotechnology Co. Ltd. (Nanjing, China).

2.1. Animals and treatment

Male Sprague-Dawley rats aged from 6 to 7 weeks (180–220 g) were purchased from Experimental Animal Center of Zhejiang province (Hangzhou, China) (Production license: SCXK 2014-0001). They were housed in Laboratory Animal Care of Life Science College under controlled temperature (22 ± 2 °C) and relative humidity (55 ± 5%) with a normal 12-h light/dark cycle. Animal welfare and experimental procedures were carried out in accordance with the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' enacted by National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) and the related ethnical regulations of Nanjing University [SYXK (SU) 2009-0017]. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Nanjing University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Rats *ad libitum* accessed a standard chow and water for one week acclimatization before the experiment. To evaluate the protection of polydatin and pioglitazone in fructose-induced liver oxidative stress, inflammation and lipid deposition, rats were randomized into the following six groups (n = 8): control vehicle, fructose vehicle, fructose with polydatin (7.5, 15, 30 mg/kg) as well as fructose with pioglitazone (positive drug, 4 mg/kg). Each rat was given drinking water or 100 mL drinking water containing 10% fructose (wt/vol) for 6 weeks, and followed by the treatment of saline injection, polydatin, or pioglitazone table by intragastric administration for next 7 weeks. All drugs were administered once daily between 2:30 p.m. and 3:30 p.m.. Animal body weight was detected weekly. Doses of polydatin and pioglitazone were selected based on our preliminary studies and other reports [22–27].