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Hypermethylated in cancer 1 (HIC1) mediates high glucose induced ROS accumulation in renal tubular epithelial cells by epigenetically repressing SIRT1 transcription

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ABSTRACT

Reactive oxygen species (ROS) is a key regulator of an array of physiological and pathological processes. While essential for the host defense mechanism, excessive ROS generation and/or deficient clearance is blamed for the pathogenesis of human diseases. In the present study, we investigated the regulatory role of hypermethylated in cancer 1 (HIC1), a transcription factor, in high glucose-induced ROS accumulation in renal tubular epithelial cells (HK-2). Treatment with high glucose (HG) not only markedly up-regulated HIC1 expression but prompted its translocation into the nucleus. HG stimulation promoted HIC1 binding to the promoter of SIRT1, a known HIC1 target with anti-oxidative ability. The recruitment of HIC1 to the SIRT1 promoter was paralleled by the enrichment of trimethylated histone H3K27 and 5-methyl cytosine, two well-characterized markers for trans-repression. HIC1 silencing with small interfering RNA abrogated SIRT1 repression by HG and at the same time weakened ROS accumulation in HK-2 cells. Knockdown or pharmaceutical inhibition of SIRT1 preempted the effect of HIC1 depletion by restoring ROS accumulation and down-regulating the expression of antioxidant genes. Mechanistically, HIC1 interacted with and recruited EZH2, an H3K27 trimethyltransferase, and DNA methyltransferase 1 (DNMT1) to repress SIRT1 transcription in response to HG stimulation. Depletion or inhibition of EZH2 or DNMT1 rescued SIRT1 expression and blocked ROS accumulation in HG-treated HK-2 cells. In conclusion, our data suggest that epigenetic repression of SIRT1 by HIC1 may contribute to HG-induced elevation of ROS levels in renal tubular epithelial cells.

1. Introduction

Modernization brings about changes in life style and dietary choices, which, combined with exposure to various environmental biohazards, collectively cultivate the epidemic of metabolic syndrome in the past decade or so [1]. Diabetes represents a prototypical form of metabolic syndrome. The development of glucose-lowering drugs is responsible for attenuating hyperglycemia in patients with diabetes. A large fraction of these patients, however, develop such complications as diabetic nephropathy and diabetic retinopathy that ultimately progress to kidney failure and blindness despite the normalization of blood glucose levels [2]. The mechanism whereby high glucose inflicted damages become cell-autonomous after the onset of the initial insult remains incompletely understood.

Among the many detrimental changes induced by high glucose is excessive accumulation of reactive oxygen species (ROS). Generally considered an indispensable part of the host defense mechanism, ROS links mitochondria function to enhanced innate immunity [3,4]. ROS also guides proper lineage specification during development [5]. Overproduction and/or insufficient clearance of ROS, on the other hand, tends to cause irreversible damages to cells and tissues eventually leading to organ failure. In the pathogenesis of diabetic nephropathy (DN), for instance, NADPH oxidase 4 (NOX4) is thought be responsible for increased ROS production in the kidneys [6]. In accordance, genetic deletion or pharmaceutical inhibition of NOX4 attenuates DN in mice. On the contrary, deficiency of superoxide dismutase 1 (SOD1), a major ROS scavenger, exacerbates renal injury in diabetic mice [7].

Silent information regulator 1 (SIRT1) is a class III lysine

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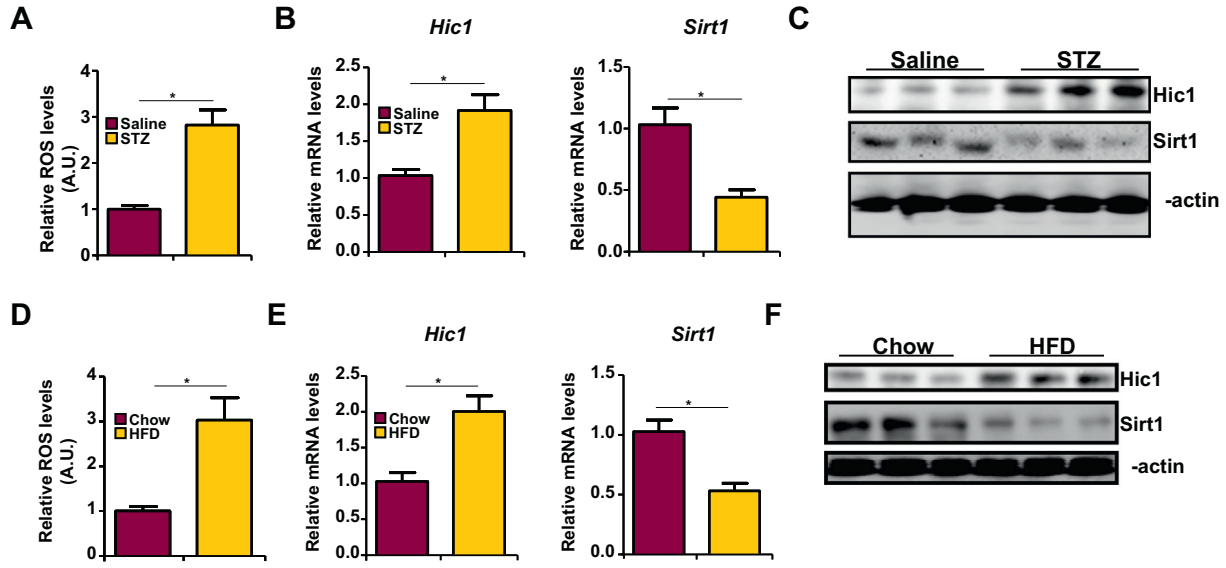

Fig. 1. HIC1 expression is up-regulated in the kidneys during DN pathogenesis in mice. (A–C) C57/BL6 mice were induced to develop diabetic nephropathy by streptozotocin (STZ) injection as described in Methods. Renal ROS levels were examined by a luminescence assay (A). HIC1 expression levels in the kidneys were examined by qPCR (B) and Western (C). (D–F) C57/BL6 mice were induced to develop diabetic nephropathy by high-fat diet (HFD) feeding as described in Methods. Renal ROS levels were examined by a luminescence assay (D). HIC1 expression levels in the kidneys were examined by qPCR (E) and Western (F). N = 4 mice for each group. Error bars represent SD (*$p < 0.05$, one-way ANOVA with *post hoc* Scheffe test).

deacetylase with ubiquitous expression and diverse function in mammalian cells [8]. SIRT1 levels are acutely influenced by stress stimuli and usually associated with the development and progression of human diseases [9,10]. One of the many protective roles SIRT1 plays in disease pathogenesis relies on its ability to curb ROS accumulation in part by promoting the up-regulation of SOD proteins [11,12]. In diabetic nephropathy, SIRT1 levels are down-regulated in the kidneys [13]. Predictably, SIRT1 over-expression or stimulation with agonists ameliorates diabetic nephropathy [14]. By comparison, SIRT1 deletion or inhibition exacerbates diabetic nephropathy [13,15,16].

Hypermethylated in cancer 1 (HIC1) is a transcription factor initially identified as a p53 target gene potentially acting as a tumor suppressor [17]. HIC1 expression is detectable in a wide range of cells including epithelial cells [18], fibroblast cells [19], lymphocytes [20], skeletal muscle cells [21], and smooth muscle cells [22], indicative of the diverse roles HIC1 plays in regulating human pathophysiology. Among the many transcriptional targets of HIC1, SIRT1 has received the most attention owing to the wide range of roles it plays regulating the metabolic homeostasis. In the present study, we investigated the epigenetic mechanism whereby HIC1 regulates SIRT1 transcription in renal epithelial cells in the context of diabetic nephropathy. Our data suggest that HIC1 may coordinate the interplay between histone and DNA methyltransferase to repress SIRT1 transcription thereby contributing to ROS accumulation to promote diabetic nephropathy.

2. Methods

2.1. Animals

All animal protocols were approved by the NJMU Intramural Ethics Committee on Animal Studies. To induce diabetic nephropathy, 6–8 week-old male C57/BL6 mice were fed with a high-fat diet (D12331, Research Diets, New Jersey, USA) for 16 weeks. Alternatively, mice were injected intraperitoneally with STZ (200 mg/kg) as previously reported and sacrificed 16 weeks following injection [23].

2.2. Cell culture and treatment

Human renal tubular epithelial cells (HK-2) were maintained in low-glucose (5.5 mM) DMEM supplemented with 10% fetal bovine serum (FBS). D-Glucose (Sigma) was added to the media bring glucose concentration to 35 mM (defined as high glucose, or HG) as previously described [23]. Mannitol (Sigma) was added to the low glucose media to control for equal osmolarity.

2.3. Plasmids and transient transfection

FLAG-tagged HIC1 and SIRT1 promoter-luciferase construct have been previously described [20,21,24]. Small interfering RNAs were purchased from Dharmacon. Transient transfections were performed with Lipofectamine 2000 (DNA plasmids) or Lipofectamine RNAiMax (siRNA). Luciferase activities were assayed using a luciferase reporter assay system (Promega).

2.4. Protein extraction and Western blot

Whole cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100) with freshly added protease inhibitor (Roche). Nuclear proteins were extracted essentially as described before [25]. Western blot analyses were performed with anti-HIC1 (Santa Cruz, sc-271499), anti-EZH2 (Proteintech, 21800-1), anti-DNMT1 (Santa Cruz, sc-271729), anti-SOD1 (Proteintech, 10269-1), anti-SOD2 (Proteintech, 24127-1), anti-SIRT1 (Abcam, ab110304), and anti-β-actin (Sigma, A5316) antibodies.

2.5. Luminescence ROS assay

Quantitative measurements of intracellular ROS were performed with a ROS-Glo system (Promega). Briefly, a luminescence substrate solution was added to and incubated with cultured cells for 6 h followed by the addition of the diction solution. Luminescence was measured using a microplate reader. Data were expressed as relative ROS levels compared to the control group.

2.6. RNA isolation and real-time PCR

RNA was extracted with the RNeasy RNA isolation kit (Qiagen). Reverse transcriptase reactions were performed using a SuperScript