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RIP3-deficience attenuates potassium oxonate-induced hyperuricemia and kidney injury

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ABSTRACT

Recent preclinical and clinical evidence suggests that hyperuricemia (HU) is an independent risk factor for metabolic syndrome, hypertension, cardiovascular disease and chronic kidney disease. Receptor-interacting protein 3 (RIP3) is an important contributor in inducing programmed necrosis, representing a newly identified mechanism of cell death combining features of both apoptosis and necrosis. In our study, RIP3 was strongly expressed in mice with hyperuricemia. RIP3 deficiency attenuated hyperuricemia in mice, evidenced by reduced serum uric acid and creatinine and enhanced urinary uric acid and creatinine, as well as the improved histological alterations in renal sections. Additionally, RIP3-deletion reduced malondialdehyde (MDA), H_2O_2 and O_2^-, whereas enhanced superoxide dismutase (SOD), GSH and GSH-Px levels in potassium oxonate-induced mice. Potassium oxonate-treated mice showed significantly high mRNA levels of ATP-binding cassette, subfamily G, membrane 2 (ABCG2), organic anion transporter 1 (OAT1), OAT3, organic cation transporter 1 (OCT1) and organic cation/carnitine transporter 1 (OCTN1) in renal tissue samples, which were reversed by RIP3-deficiency. Meanwhile, down-regulation of circulating and kidney pro-inflammatory cytokines (IL-1β, TNF-α and IL-6) were observed in RIP3-knockout mice with hyperuricemia, associated with inactivation of toll-like receptor 4 (TLR4), inhibitor of NF-κB alpha (IκBα) and nuclear factor kappa B (NF-κB). NLR family, pyrin domain-containing 3 (NLRP3) inflammasome was also suppressed by RIP3 knockout in potassium oxonate-treated mice. Importantly, RIP3-knockout mice exhibited the decrease of FAS-associated protein with a death domain (FADD), cleaved Caspase-8/-3 and Poly (ADP-ribose) polymerase (PARP) in renal samples, along with TUNEL reduction in mice with hyperuricemia. Similar results were observed in uric acid-incubated cells with RIP3 knockdown. Thus, we suggested that RIP3 played an important role in mice with hyperuricemia, which might be a novel signal pathway targeting for therapeutic strategies in future.

1. Introduction

Hyperuricemia is characterized by a persistent increase of serum urate concentrations, in which monosodium urate monohydrate crystals may be deposited in tissues [1–3]. UA (uric acid) is the final product of purine metabolism in humans and is implicated in many disease conditions [4]. Presently, various pathways have been reported to modulate the pathogenesis of hyperuricemia. Increasing evidences demonstrate that UA-induced oxidative stress and inflammation are the essential mechanisms for renal injury in hyperuricemic rodents and human [5]. Recent research suggests that hyperuricemia may be caused by the elevated activity of the enzyme xanthine oxidase (XO). Additionally, UA can react with O_2^-, H_2O_2, and hydroxyl radical (OH^-) to promote the oxidative stress and kidney dysfunction [6]. TLR4/NF-κB is a key transcription pathway to modulate the secretion of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6, contributing to renal injury [7]. However, hyperuricemia-associated disease progression is not completely understood.

Apoptosis has been considered as a possible target for novel therapies in various diseases [8]. Recently, a novel mechanism, named as necroptosis, has been reported as another essential regulator of cell death [9]. Similar to apoptotic cell death, the process is tightly modulated by distinct molecules, but results in the typical morphological signs of necrosis, including defects of inflammation and membrane integrity, therefore combining features of both mechanisms [10]. The signal pathway activating programmed necrosis is not fully investigated. RIP3 is an essential step for inducing programmed necrosis [11]. RIP3-regulated necroptosis has been indicated during viral infection of liver, high fat diet-induced damage in liver and in tissue injury due to inflammatory bowel disease [12]. However, the role of RIP3 in the context of hyperuricemia is still unknown.

In the present study, we illustrated that RIP3 was strongly expressed

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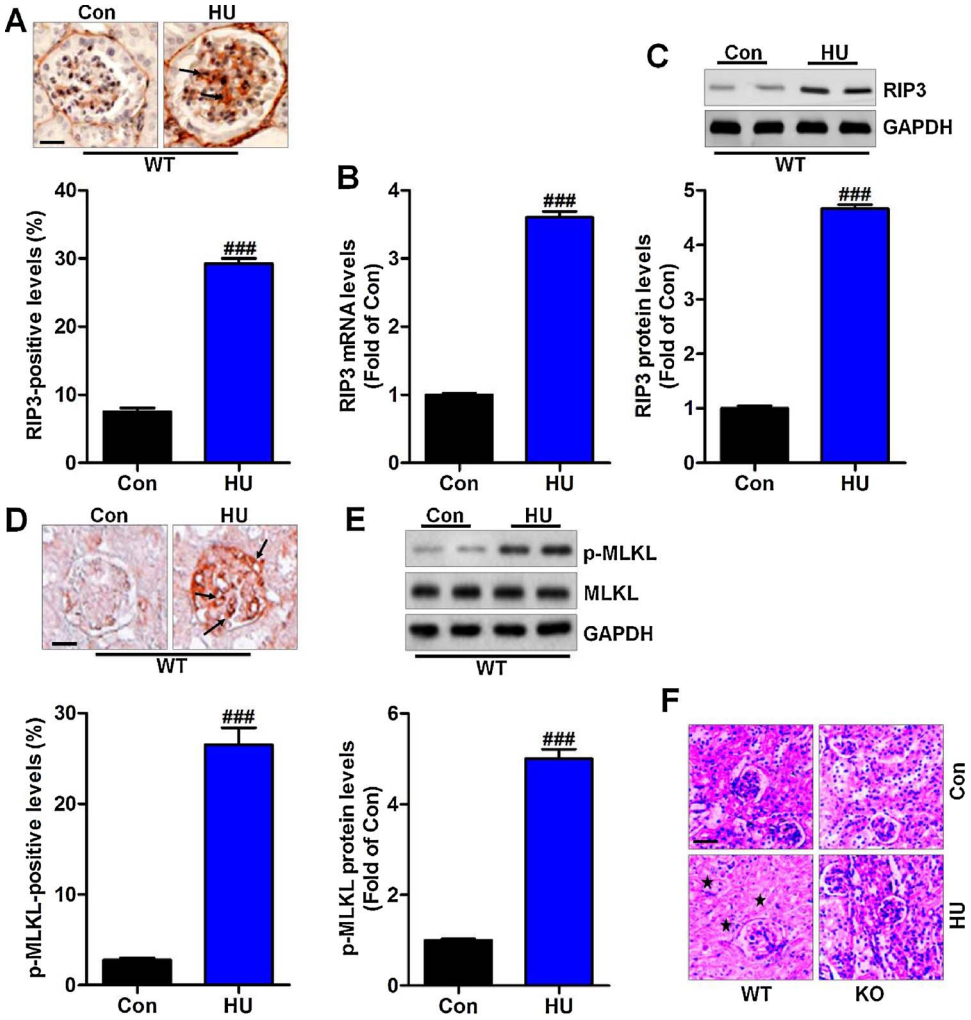

Fig. 1. Potassium oxonate increases RIP3 expression in kidney of mice. (A) IHC analysis of RIP3 in renal sections. (B) RT-qPCR and (C) western blot assays of RIP3 expressions in kidney. (D) IHC staining of p-MLKL in kidney sections. (E) Western blot analysis of renal p-MLKL. (F) H&E staining of renal sections isolated from each group of mice. The red star refers the abnormal histological changes of renal samples. Data were shown as mean ± S.E.M. n = 6. $^{###}P < .001$ vs. Con group.

in renal tissue samples in mice with hyperuricemia. And RIP3 knockout and knockdown significantly attenuated kidney injury, along with reduced oxidative stress, inflammation and apoptosis in vivo and in vitro. Our study provided a novel target, RIP3, which was essential for hyperuricemia progression. And it could be a promising candidate for investigating effective therapeutic strategies in future.

2. Materials and methods

2.1. Animals

The wild type (WT) and RIP3-knockout (#025738, KO) 6 week-old C57BL/6J male mice weighing 18–20 g were purchased from Jackson Laboratory (Bar Harbor, ME). They were allowed 1 week to adapt to their environment before experiments. They were housed under a normal 12 h/12 h light/dark schedule at room temperature (22 ± 2 °C) with relative humidity (55 ± 5%), and given a standard chow and water ad libitum. All procedures were in line with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People's Republic of China. The Institutional Animal Care and Use Committee at The second Affiliated Hospital, School of Medicine, Zhejiang University (China) approved the animal study protocols.

All mice were divided into 4 groups (n = 6/group): 1) WT-Con; 2) KO-Con; 3) WT-HU (potassium oxonate-induced hyperuricemia); and 4) KO-HU. Potassium oxonate (250 mg/kg) was administered to mice through oral gavage once a day for 7 consecutive days. The 24 h urine was collected and centrifuged with 2000*g* at 4 °C for 10 min for removing particulate contaminants. The urine was collected 1 h after final administration on the 7th day for further assays. Mice were then scarified by final blood withdrawal. Samples were collected by retrobulbar puncture and then were placed at 37 °C for 30 min. Next, the blood samples were centrifuged (3000 × *g*) at 4 °C for 10 min to isolate serum, which was immediately frozen at −80 °C for biochemical assays. The renal tissue samples were harvested for fixing in 4% formalin for immunohistochemical analysis, or snap-frozen in liquid nitrogen and stored at −80 °C for western blot and RT-qPCR assays. Both approaches (formalin fixation and snap-freezing) were applied to tissue samples of each animal.

2.2. Determination of biochemicals

BUN (Urea Assay Kit), UA (uric acid Assay Kit) and creatinine (creatinine Assay Kit) in serum or kidney were measured using biochemical kits (Nanjing Jiancheng Biotechnology, China). MDA (Malondialdehyde (MDA) assay kit (TBA method)), SOD (Superoxide Dismutase (SOD) assay kit (WST-1 method)), GSH (glutathione (GSH) assay kit) and GSH-Px (Glutathione Peroxidase (GSH-PX) assay kit) activity were also calculated with commercially available kits (Nanjing Jiancheng Biotechnology). Renal H_2O_2 (Hydrogen Peroxide Assay Kit)