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P53 modulates hepatic insulin sensitivity through NF-κB and p38/ERK MAPK pathways

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ABSTRACT

Besides its well-established oncosuppressor activity, the role of p53 in regulating metabolic pathways has been recently identified. Nevertheless, the function of p53 with respect to insulin resistance appears highly controversial. To address this issue, we investigated the expression of p53 in experimental model of insulin resistance. Then we used activator (nutlin-3α) and inhibitor (pifithrin-α, PFT-α) of p53 in HepG2 cell. Here we showed that p53 protein level was decreased in the hepatic tissue of high-fat diet-induced insulin resistance mice, genetically diabetic ob/ob mice and palmitate (PA) treated HepG2 cells. And high expression of phosphor-p38, ERK1/2 and nuclear factor kappa B (NF-κB) p65 accompanied with low expression of p53. But activation of p53 with nutlin-3α prevented PA-induced reduction of glucose consumption and suppression of insulin signaling pathways. At the same time, nutlin-3α downregulated the activation of NF-κB, p38 and ERK1/2 pathways upon stimulation with PA. In contrast, inhibition of p53 with PFT-α decreased glucose consumption and suppressed insulin signaling pathway. Furthermore, PFT-α activated NF-κB, p38 and ERK1/2 pathways in HepG2 cells. Overall, these results suggest that p53 is involved in improving insulin sensitivity of hepatic cells via inhibition of mitogen-activated protein kinases (MAPKs) and NF-κB pathways.

1. Introduction

Insulin resistance (IR), defined as impairment of reactivity to physiological concentrations of insulin, is significantly correlated with several metabolic diseases such as type 2 diabetes mellitus (T2DM), obesity and metabolic syndrome [1]. Emerging evidence indicates that inflammation and oxidative stress contributes to the development of IR [2,3].

P53 gene has been declared the major tumor suppressor gene. Interestingly, p53 presence seems to be vastly connected with metabolic abnormalities underlain by inflammation, oxidative stress, cellular aging and obesity [4]. Accumulating amount of data identifies p53 to be a factor activated upon excessive calorie intake or fat intake, thus contributing to a low-grade chronic inflammation and systemic insulin resistance [5,6]. Increased level of p53 was also reported in a streptozotocin-induced diabetes rats [7] and genetically obese (ob/ob) mice [8]. When selective p53 transcriptional activity inhibitor called pifithrin-α (PFT-α) was administered to high-fat diet feed mice, insulin action was improved [5]. Prominent sighs of p53 actions were observed in liver, muscles and adipose tissue being associated with attenuation of insulin signaling [9]. Prokesch et al. reported that the liver p53 level is required for gluconeogenesis [10].

While other studies have suggested p53 play a potential important role in controlling glucose homeostasis. The lowered serum level of TP53 gene product was reported in diabetic subjects as well as patients with impaired glucose tolerance [11]. In p53 heterozygote knockout mice, reduced p53 expression caused glucose intolerance in response to a Western type diet [12]. In contrast, overexpression of p53 is sufficient to improve glucose homeostasis. In a type 2 diabetic mouse model produced by high-fat diet combined with streptozotocin (STZ) injections, activation of p53 by transfection of the recombinant adenoviral p53 led to an stable expression of p53 in liver and pancreas tissues and result in

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antidiabetic effect [13]. Similarly, it has been reported that Nutlin-3α, an activator of the p53 pathway, also ameliorates the severity of STZ-induced DM in mice [14]. Additionally, it was revealed that mice with relative deficits in the transactivation domain of p53 exhibit defects in glucose homeostasis, while mice with an increased dosage of p53 exhibit improved glucose tolerance [15]. These contradictory data reflects the need for further confirmation of the p53 role in the regulation of insulin sensitivity.

In previous study, we found that the p53 expression was decreased in liver of high-fat diet-induced obese mice and ob/ob mice. To gain better insight to the effect of p53 in IR, this study investigated the expression of p53 in palmitic acid (PA) induced IR model in HepG2 cells. Furthermore, we examined the effect of activator and inhibitor of p53 on insulin sensitivity in HepG2 cells.

P53 is entangled in a broad crosstalk with inflammatory elements, such as NF-κB, mitogen-activated protein kinases (MAPKs) pathways and ROS. It was reported p53 attenuate inflammation through inhibition of NF-κB pathway [16,17], as lack of p53 results in NF-κB activation [18]. p53 also plays a role in MPAKs signaling pathways, which regulates insulin sensitivity and inflammation [19]. Therefore, this research analyzed the influence of p53 on the expression of NF-κB and MAPKs pathways in HepG2 cells.

2. Materials and methods

2.1. Animal models of high fat diet induced insulin resistance

The animal protocol had been approved by the Institutional Animal Care & Use Committee (IACUC) of Nanjing Medical University (Protocol Number NJMU08-092). Male C57BL/6 J mice (5 weeks old) were purchased from the Animal Center of Nanjing Medical University (Nanjing, Jiangsu, China) and housed in a specific pathogen-free facility throughout the experimental period. The mice were maintained at 22−24 °C on a 12-h-light/dark cycle with ad libitum access to water and food. After 1-week acclimatization period, the mice were randomly divided into 2 groups (n = 6 per group): normal diet (control), high fat diet (HFD). The normal diet was a purified diet based on the AIN-76 rodent diet composition. The HFD was identical to the control, but to which 200 g fat/kg (170 g lard plus 30 g corn oil) and 1% cholesterol had been added.

After 11 weeks, for glucose tolerance test (GTT), the mice were fasted for 12 h and injected intraperitoneally with glucose (1 g/kg body weight). The level of blood glucose was measured in tail blood samples at 0, 30, 60 and 120 min after glucose injection using test strips on Contour®TS [Bayer Diabetes Care, Basel, Switzerland].

After 12 weeks, the mice were anesthetized with diethyl ether after an overnight fasting for 12 h, and their blood samples were collected. Serum samples were isolated by centrifugation at 3000 × g for 10 min and stored at −80 °C for insulin analysis. Liver tissues were collected, washed with phosphate-buffered saline, and frozen at −80 °C.

2.2. Animal models of genetical insulin resistance

Six-week-old male leptin null mutant mice *(ob/ob* mice*)* and age-matched control mice (C57BL/6 mice) were used for the study. Mice were given access to normal diet and water ad libitum and housed on a 12-h light/dark cycle. After 12 weeks, Body weight was measured and the level of 12 h-fasting blood glucose was measured by using test strips on Contour®TS [Bayer Diabetes Care, Basel, Switzerland] and then these mice were sacrificed. Both liver and blood of these mice were collected.

2.3. ELISA

The concentrations of serum insulin in individual mice were determined using the mouse insulin ELISA kit, according to the manufacturer's instructions (R & D Systems, Minneapolis, Minn., USA). Absorbance of standards and samples were determined spectrophotometrically using a microplate reader (TECAN, Austria).

2.4. Cell culture

The HepG2 cell line, that is used commonly as a hepatocyte model for studies of insulin resistance [20,21], was obtained from Chinese Academy of Typical Culture Collection Cell Bank. The logarithmically growing cells were used in the following experiment. Palmitate acid, nutlin-3 and PFT-α were dissolved in DMSO, control cells were treated with matching concentrations of DMSO. The HepG2 cells were treated with palmitate and/or nutlin-3α, pifithrin-α(PFT-α).

2.5. MTT

HepG2 cells were seeded in 96-well plates at a density of 3×10^4 cells/well in 100 μl of medium. Twenty-four hours later, the medium was replaced and cells were treated with various concentrations of PA for 24 h. After treatment, cell viability was then assessed using MTT assay. Absorbance was measured at 570 nm using a microplate reader (TECAN, Austria).

2.6. Glucose consumption

After treatment, HepG2 cells were washed with and cultured in MEM. Insulin (10 nM; Novo Nordisk) was added thereafter and incubated for another 1 h. Glucose concentration in the culture supernatant was determined with the glucose assay kit (Nanjing Jiancheng Bioengineering Institute, China). Glucose consumption was calculated by using the initial glucose level and the remaining glucose level in the supernatant.

2.7. Western bloting

The liver tissue samples were obtained from individual mice and lyzed in NP40 lysis buffer, followed by centrifugation. The treated HepG2 cells were washed twice with PBS and lysed in responsive lysis buffer, followed by centrifugation. After quantification of protein concentrations, the liver or cell lysates (20−30 μg of protein) were separated by SDS-PAGE on 10% polyacrylamide gels, and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA). The membranes were blocked using either 5% non-fat dried milk (for total proteins) or BSA (for phospho-specific antibodies) and incubated with anti-IR, anti-IRS, anti-*p*-AKT (Affinity Biosciences, Victoria, USA), anti-p53, anti-p-p65, anti-*p*-ERK1/2 (Thr202/Tyr204), anti-p-p38 (Thr180/Tyr182) (Cell Signaling Technology, Danvers, USA), and anti-GAPDH (Bioworld, Shanghai, China) respectively. The bound antibodies were detected using horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using enhanced chemiluminescence (ECL, Millipore). The relative levels of target proteins to controls were determined by densimetric analysis using ImageJ software.

2.8. Statistical analysis

Statistical analyses were performed with SPSS 16.0 (SSPA, Inc., Chicago, IL, USA). All data is expressed as means ± standard deviation. Student's *t*-test was used for statistical evaluation between the two groups. One-way ANOVA was used for the comparison of

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