Mst1 regulates hepatic lipid metabolism by inhibiting Sirt1 ubiquitination in mice

Chao Geng a, Yinliang Zhang a, Yong Gao a, Wufan Tao b, Huabing Zhang c, Xiaojun Liu a,*, Fude Fang a,***, Yongsheng Chang c,***

a National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences & School of Basic Medicine Peking Union Medical College, #5 Dongdansantiao, Beijing 100005, China
b State Key Laboratory of Genetic Engineering and Institute of Developmental Biology, Collaborative Innovation Center of Genetics and Development, School of Life Sciences, Fudan University, Shanghai 200433, China
c Department of Biochemistry & Molecular Biology, School of Basic Medicine, Anhui Medical University, Hefei 230032, China

A R T I C L E   I N F O

Article history:
Received 1 February 2016
Accepted 15 February 2016
Available online 20 February 2016

Keywords:
Mst1
Sirt1
Non-alcoholic fatty liver disease
Oxidant stress

A B S T R A C T

Previous study showed mammalian Ste20-like kinase (Mst1) may serve as target for the development of new therapies for diabetes. However, the function of Mst1 involved in liver lipid metabolism has remained elusive. In this study, we report that the liver of Mst1 knockout (Mst1−/−) mice showed more severe liver metabolic damage under fasting and high-fat diet than that of control mice. And fasting induced hepatic Mst1 expression. Mst1 overexpression inhibited Srebp-1c expression and increased the expression of antioxidant genes in primary hepatocytes. We also found that fasting-induced expression of hepatic Sirt1 was attenuated in Mst1−/− mice. Mst1 overexpression promoted Sirt1 expression, probably due to inhibiting Sirt1 ubiquitination. In summary, our study suggests that Mst1 regulates hepatic lipid metabolism by inhibiting Sirt1 ubiquitination in mice.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

With the increase in the global obesity population, the incidence of non-alcoholic fatty liver disease (NAFLD) is expected to increase. NAFLD is defined as fat accumulation in greater than 5% of liver cells known as hepatocytes [1]. NAFLD represents a spectrum of hepatic pathologies, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis [2]. Individuals with NASH are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma [3]. The molecular pathogenesis of NAFLD is complex and involves multiple genetic and environmental factors. Some studies have indicated that the pathogenesis of NAFLD is characterized by the ‘two hit’ model [4]. The first hit occurs when insulin resistance causes lipid accumulation in hepatocytes, and the second hit occurs when cellular insults, resulting in inflammation and fibrosis from oxidative stress, mitochondrial dysfunction, lipid peroxidation and cytokine activity [5]. However, there is increasing evidence that free fatty acids can directly cause toxicity by increasing oxidative stress and by activation of inflammatory pathways, therefore hepatic triglyceride accumulation may be a protective mechanism by preventing the toxic effects of unesterified FFA [6]. Additionally, the development of fibrosis/cirrhosis is dependent on the efficacy of hepatocyte regeneration and oxidative stress inhibits the replication of mature hepatocytes which replace the dead cells and reconstitute normal tissue function, therefore cell death with impaired proliferation of hepatocyte progenitors represents the proposed third hit in NAFLD pathogenesis [6].

The mammalian Ste20-like kinases (Msts), of which Mst1 and Mst2 share the highest degree of homology and are ubiquitously expressed serine–threonine kinases. Mst1 is part of the Hippo signaling pathway and involved in multiple cellular processes such as morphogenesis, proliferation, stress response and apoptosis [7,8]. Mst1 can phosphorylate the FoxO transcription factors at a site that is conserved within the forkhead domain of these proteins from mammals to C. elegans. Sirt1 is a nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylase and serves as an energy sensor to modulate insulin sensitivity through its substrates,
most importantly FoxO1 [9]. Previous study demonstrated that Mst2 and Salvador homolog 1 (Sav1), a scaffolding protein that functions in the Mst pathway, significantly increased PPARγ trans-activation and augmented differentiation of 3T3-L1 cells, indicating PPARγ activation by the Mst signaling pathway may be a novel regulatory mechanism of adipogenesis [10]. Recently, Ardestani et al. reported Mst1 as a proapoptotic kinase and key mediator of apoptotic signaling and beta cell dysfunction and suggest that Mst1 may serve as target for the development of new therapies for diabetes [11]. However, the function of Mst1 involved in liver lipid metabolism has remained elusive.

In this study, we report that HFD aggravated liver metabolic damage in Mst1−/− mice. In primary hepatocytes, Mst1 overexpression promoted Sirt1 expression by inhibiting Sirt1 ubiquitination, furthermore decreased Srebp-1c expression and improved antioxidant genes expression. Our results suggest that Sirt1 mediates the effect of Mst1 on hepatic lipid metabolism.

2. Materials and methods

2.1. Animal protocols

Mst1-deficient (Mst1−/−) mice and wild-type 129 mice were provided by Professor Tao (Fudan University, China). Generation of Mst1−/− mice was previously reported [12]. All mice were housed and maintained on a 12 h light–dark cycle and a regular unrestricted diet. The mice were fed with either a normal chow (9% fat; Lab Diets) or HFD (45% fat; Research Diets) and libitum with free access to water. The diagnosis of nonalcoholic steatohepatitis (NASH) is defined by the presence and pattern of specific histological abnormalities on liver biopsy according to NAFLD Activity Score (NAS) [13]. All animal experiments were conducted under protocols approved by the Animal Research Committee of the Institute of Laboratory Animals, Chinese Academy of Medical Sciences and Peking Union Medical College. All the surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Histological analysis

For H&E staining, liver tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and cut into 7-μm sections. For Oil Red O staining, liver tissue was frozen in liquid nitrogen and cut into 10-μm sections. Sections were stained and analyzed at 20 × magnification using a microscope.

2.3. Cell culture

Primary hepatocytes were isolated and cultured as previously reported [14]. Mouse hepatocytes were infected by adenovirus expressing His-tagged Mst1. 293A cells were grown at 37°C, 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 1% (v/v) penicillin-streptomycin. Cell transformation was performed according to the manufacturer’s protocol (Vigorous).

2.4. Western blotting

Protein was extracted from frozen liver samples or cultured hepatocytes in cell lysis buffer. In total, 40–60 μg of protein was loaded onto a 10% SDS-polyacrylamide gel, and separated proteins were transferred to PVDF membranes. Western blot assays were performed using specific antibodies. Anti-Sirt1 antibody was purchased from Cell Signaling Technologies (Beverly, MA). Anti-Ho-1 and -Sod2 antibody were from Abclonal Technology. Anti-α-tubulin was obtained from Abmart (Arlington, MA).

2.5. RNA isolation and quantitative RT-PCR

Total RNA was extracted from the primary hepatocytes using a TRIzol-based method (Roche). Approximately 2 μg of total RNA was reverse-transcribed into a first-strand cDNA pool using Super-Script™ reverse transcriptase and random primers (Abcam). Quantitative real-time reverse-transcriptase PCR was performed using the SYBR Green I Q-PCR kit (Promega) on a Bio-Rad CFX system. All gene expression data were normalized to β-actin expression levels. Primer sequences are available upon request.

2.6. Statistical analysis

All the data are indicated as mean ± s.d. Differences among means are analyzed by Independent-sample T test. p < 0.05 was considered statistically significant.

3. Results

3.1. Fasting and high-fat diet aggravate liver metabolic damage in Mst1−/− mice

To study the function of Mst1 gene in liver metabolism, we first analyzed the liver phenotypic response of Mst1−/− mice to fasting. We found that fasting increased hepatic lipid accumulation and showed hepatic steatosis in the control mice (Fig. 1A), however, the liver damage was aggravated in Mst1−/− mice, displaying accumulations of lipid droplets and degeneration of ballooning in liver (Fig. 1A). Then, we evaluated the liver damage when Mst1−/− mice were fed by HFD for 24w. The results showed that Mst1−/− mice on HFD displayed much worse phenotypes while 129 mice on HFD presented with apparent hepatosteatosis, including massive accumulations of large lipid droplets and ballooning degeneration of liver cells (Fig. 1B).

3.2. Mst1 decreases Srebp-1c expression and increases the expression of antioxidant genes

Though fasting induces the change of activation and expression of many genes, we examined the effect of fasting on Mst1 expression. The results showed that fasting induced the mRNA expression of Mst1 in mouse livers (Fig. 2A). Lipid accumulation in the liver is a major hallmark of NAFLD and Srebp-1c, a key transcription factors regulating hepatic lipid metabolism, has been proposed to have great potential for NAFLD treatment [15]. Collective studies have suggested that oxidative stress may also contribute to clinical progression from simple fatty liver to NASH [16]. So, we studied the impact of Mst1 on Srebp-1c and some antioxidant genes. Our results showed that Mst1 overexpression decreased the mRNA level of Srebp-1c, and increased the mRNA level FoxO1, Ho-1, Sod2, Gpx1 and Gpx1 in mouse primary hepatocytes infected with Ade-Mst1 (Fig. 2B). In addition, we found that Mst1 overexpression increased the protein expression level of Ho-1 and Sod2 (Fig. 2C) and reduced mitochondrial ROS production in WT primary hepatocytes (Fig. 2D).

3.3. Fasting-induced hepatic Sirt1 increase is attenuated in Mst1−/− mice

Previous studies have indicated that fasting induced the expression of Sirt1 and activation of Sirt1 ameliorated hepatic steatosis by inhibiting Srebp-1c expression and increasing antioxidant capacity in the liver [17–21], thus we examined whether the
Sirt1 expression of induced by fasting was affected in Mst1−/− mice. As previous reported, our result showed that fasting induced hepatic Sirt1 expression in 129 mice (Fig. 3). However, the effect of fasting-induced hepatic Sirt1 increase was attenuated in Mst1−/− mice (Fig. 3). These results indicate that Mst1 may involve in fasting-induced Sirt1 expression.

3.4. Mst1 increases the expression of Sirt1 by inhibiting Sirt1 ubiquitination

To further explore the effect of Mst1 on Sirt1, we performed the following experiments. The result showed that Mst1 over-expression enhanced Sirt1 expression in primary hepatocytes (Fig. 4A) and 293A cells (Fig. 4B). However, Mst1K59R, a kinase-dead
Mst1 in which the ATP binding site was mutated, could not increase the expression of Sirt1 in 293A cells (Fig. 4B). Furthermore, we found overexpression of Mst1, but not Mst1K59R, inhibited Sirt1 ubiquitination in 293A cells (Fig. 4C). Moreover, our study revealed that the Mst1 protein was co-immunoprecipitated with Sirt1, further confirming interaction between Mst1 and Sirt1 proteins (Fig. 4D).

4. Discussion

Previous studies have indicated Mst1 as a proapoptotic kinase and key mediator of apoptotic signaling and beta cell dysfunction and suggested that it may serve as a target for the development of new therapies for diabetes [11]. However, the role of Mst1 in liver metabolism remains unclear. The minimal histologic criteria for the diagnosis of NASH include fat accumulation and the presence of hepatocyte swelling or ballooning degeneration and lobular inflammation [22]. A separate histologic scoring system for the spectrum of NAFLD, called the NAFLD Activity Score (NAS), was developed as a tool to measure changes in NAFLD [13,23,24]. In this study, we found that both of fasting and high-fat diet cause fat accumulation in the liver of 129 mice, and this phenomenon was aggravated in the liver of Mst1−/− mice. In addition, Mst1−/− mice also displayed hepatocyte swelling or ballooning degeneration and lobular inflammation in the liver. It is now well known that fasting causes a rapid transcriptional activation of genes encoding mitochondrial, peroxisomal and microsomal fatty acid oxidation in liver in healthy individuals [25,26]. Mst1 activity was highly upregulated upon chronic exposure to increasing glucose concentrations or palmitic acid, or upon exposure to acute oxidative stress from hydrogen peroxide [11]. In this study, we show that fasting induces Mst1 in which the ATP binding site was mutated, could not increase the expression of Sirt1 in 293A cells (Fig. 4B). Furthermore, we found overexpression of Mst1, but not Mst1K59R, inhibited Sirt1 ubiquitination in 293A cells (Fig. 4C). Moreover, our study revealed that the Mst1 protein was co-immunoprecipitated with Sirt1, further confirming interaction between Mst1 and Sirt1 proteins (Fig. 4D).

Srebp-1c has been identified as a key transcriptional regulator involved in liver lipid homeostasis [27]. Our result demonstrated that Mst1 overexpression inhibited Srebp-1c expression in primary hepatocytes. Srebp-1c levels were elevated in the fatty livers of different animal models including obese, insulin-resistant and hyperinsulinemic ob/ob mice and were also depressed during fasting but increased markedly when animals were refed a high carbohydrate diet [28]. Collective studies have indicated that oxidative stress and mitochondrial dysfunction play an important role in the pathogenesis of NAFLD [6]. Oxidative stress induces Srebp-1c activation and liver steatosis, the mechanisms of this process likely include an activation of the endoplasmic reticulum stress pathway [28]. In addition, β-oxidation taking place in the mitochondria can become overwhelmed in NAFLD and give rise to excessive ROS. Excessive ROS induce oxidative stress, activate inflammatory pathways and damage mitochondrial function, eventually lead to steatohepatitis and/or fibrosis [6]. So, antioxidant strategy as a new therapeutic direction may prevent the evolution of metabolic diseases, such as NAFLD and type 2 diabetes. In this study, we found that both of fasting and high-fat diet cause fat accumulation in the liver of 129 mice, and this phenomenon was aggravated in the liver of Mst1−/− mice. In addition, Mst1−/− mice also displayed hepatocyte swelling or ballooning degeneration and lobular inflammation in the liver. It is now well known that fasting causes a rapid transcriptional activation of genes encoding mitochondrial, peroxisomal and microsomal fatty acid oxidation in liver in healthy individuals [25,26]. Mst1 activity was highly upregulated upon chronic exposure to increasing glucose concentrations or palmitic acid, or upon exposure to acute oxidative stress from hydrogen peroxide [11]. In this study, we show that fasting induces
study, Mst1 overexpression increased the expression antioxidant genes, furthermore decreased ROS production. Previous study indicated that Mst1 has an important role in diverse biological processes including cellular responses to oxidative stress and longevity [29]. In addition, peripheral T cells from both Mst1-deficient human and mice display decreased FoxO protein levels [12,30,31]. Consistently, the FoxO targets, Sod2 and catalase, were deficient human and mice display decreased FoxO protein levels [28]. Longevity [29]. In addition, peripheral T cells from both processes including cellular responses to oxidative stress and [12,30,31]. Consistently, the FoxO targets, Sod2 and catalase, were investigated that Mst1 differentially regulated TLR3/4/9-mediated inflammatory responses in macrophages and thereby is protective against chronic inflammation-associated hepatocellular carcinoma [32]. It is well known that the presence of steatosis is tightly associated with hepatic chronic inflammation, an effect in part mediated by activation of the Ikk-/NF-kB signaling pathway [6]. And the chronic inflammatory state associated with hepatic steatosis may also play a key role in hepatocellular carcinoma development.

Sirt1 has emerged as a key metabolic sensor in various metabolic tissues. In response to different environmental stimuli, Sirt1 directly links the cellular metabolic status to the chromatin structure and the regulation of gene expression, thereby modulating a variety of cellular processes such as energy metabolism and stress response [33]. In addition, calorie restriction can increase the NAD+/NADH ratio in cells and result in activation of Sirt1, furthermore extends life spans in a wide variety of species [34]. Previous study also showed that Sirt1 protein levels were induced after fasting and returned to nearly control levels upon refeeding [21]. In this study, the effect of fasting-induced hepatic Sirt1 increase was attenuated in Mst1−/− mice. Above these results indicate Mst1 involved in fasting-induced Sirt1 expression in the liver. Furthermore, our results showed that Mst1 overexpression enhanced Sirt1 expression, probably due to Mst1-mediated inhibition of Sirt1 ubiquitination. Previous studies have demonstrated that Mst1 involved in some proteins ubiquitination [11,35]. In addition, Yuan et al. reported that Mst1 can phosphorylate Sirt1 in vitro and in vivo [36]. Reversible posttranslational modification is a versatile way to regulate protein activity and crosstalk between different types of posttranslational modification has become prominent more in eukaryotic biology. Particularly prominent are the multiple connections between phosphorylation and ubiquitination, which act either positively or negatively in both directions to regulate these processes [37]. Previous studies have indicated that Sirt1 can ameliorate hepatic steatosis by inhibiting Sreb1c expression and increasing antioxidant capacity in the liver [17–20]. In this study, we report that Mst1 involved in hepatic lipid metabolism by decreasing Sreb1c expression and increasing antioxidant genes expression, which may be mediated by Sirt1.

Acknowledgments

This work was supported by collective grants from the National Natural Science Foundation of China (Grants No. 30800389, 81170763 and 31371191), National Basic Research Program of China (2013CB945304) and Natural Science Foundation of Anhui Province (1408083MH153). We thank Prof. Zengqiang Yuan for the plasmids of pCMV-MST1-Flag and pCMV-MST1K59R-Flag, and Prof. Yi Li for the expression plasmid of pE-F-IRE-P-HA-Ub.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.02.059

References

[21] G. Musso, R. Gambino, M. Cassader, Recent insights into hepatic lipid


