Research Article

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Hepatic menin recruits SIRT1 to control liver steatosis through histone deacetylation

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Background & Aims: The development and progression of non-alcoholic fatty liver disease are associated with aging, obesity, and type 2 diabetes. Understanding the precise regulatory networks of this process will contribute to novel therapeutic strategies.

Methods: Hepatocyte-specific *Men1* knockout mice were generated using Cre/loxP technology. Lipid and glucose metabolic phenotypes and mechanisms were investigated in aging and high-fat diet fed mice.

Results: The expression of menin, encoded by multiple endocrine neoplasia 1 (*Men1*) gene, is reduced in the liver of aging mice. Hepatocyte-specific deletion of *Men1* induces liver steatosis in aging mice. Menin deficiency promotes high-fat diet-induced liver steatosis in mice. Menin recruits SIRT1 to control hepatic *CD36* expression and triglyceride accumulation through histone deacetylation.

Conclusions: Our work reveals that the adaptor protein menin is critical for the progression of hepatic steatosis during aging and metabolic imbalance.

Keywords: Menin; SIRT1; Aging; Liver steatosis; Histone deacetylation.
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Abbreviations: NAFLD, non-alcoholic fatty liver disease; MEN1, multiple endocrine neoplasia type 1; LMKO, liver-specific *Men1* knockout mice; TG, triglyceride; TC, total cholesterol; HFD, high-fat diet; IPGTT, intra-peritoneal glucose tolerance tests; ITT, insulin tolerance tests; Scd-1, stearoyl CoA desaturase-1.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasing common hepatic disorder worldwide. NAFLD begins with simple hepatic steatosis and may progress to steatohepatitis, which can lead to liver fibrosis and cirrhosis [1]. The progression and increasing prevalence of NAFLD are associated with age in the general population. Age-related metabolic changes in the liver and other organs, accompanied by fat deposition and gradual weight gain, systematically contribute to the development of NAFLD. All characteristics of metabolic syndrome, specifically obesity and type 2 diabetes, are relevant to NAFLD [2,3]. The complex and dynamic transcriptional regulatory networks, which connect aging, metabolic homeostasis and pathogenesis of NAFLD, need to be further elucidated.

Recent studies have revealed that several regulatory factors in metabolic homeostasis play important roles in age-associated liver disorder [4–6]. SIRT1, a nuclear NAD^+-dependent deacetylase, is a critical regulator in various metabolic processes and aging [7,8]. Previous studies indicate hepatic SIRT1 expression links nutrient status to metabolic homeostasis, although contradictory conclusions were drawn [9–11]. Gain-of-function and loss-of-function studies using transgenic animals have revealed the functions of SIRT1 in lipid and glucose metabolism, however, the conclusions were still controversial [11–14]. Moreover, the effects of active and negative regulators of SIRT1 on liver steatosis were investigated. Activation of SIRT1 by resveratrol treatment or *DBC1* knockout was able to protect against the development of HFD-induced liver steatosis [10,15]. During aging, the expression of *Sirt1* is reduced in mouse livers [5]. Overexpression of SIRT1 improves healthy aging in mice [16]. Taken together, these elegant studies have revealed the importance and complexity of a regulatory network in the aging-related metabolic process. Novel regulatory factors, translational and post-translational regulation mechanisms in hepatic metabolic homeostasis should be further investigated for a better understanding of the uncharted and controversial issues.

In this study, we show that the expression of *Men1* (multiple endocrine neoplasia type 1) gene was reduced in the livers of aged mice. The protein product of the *Men1* gene, menin, is a functional adaptor protein in endocrine tumors, leukemia, and bone metabolism [17–19]. Menin interacts with many proteins, such as NF-κB, PPARγ and β-catenin, to control multiple biological processes [20–22]. Menin can recruit several histone modifiers, including MLL and HDACs, to regulate gene transcription via histone methylation and acetylation [23,24]. Our recent study reported that high blood glucose repressed expression of *Men1* in pancreatic β-cells [25]. In addition, insulin had been shown to suppress *Men1* expression in the liver [26]. Therefore, the expression of hepatic menin may be affected by insulin and glucose in diabetic mice [26,27]. In human studies, patients with germline mutations of *Men1* have been reported to have elevated serum TG and total cholesterol (TC) level, and have higher prevalence of cardiovascular disease and type 2 diabetes [28–30]. However, the physiological functions of hepatic menin in metabolic homeostasis are still less known.

Here we provide functional evidence that hepatic menin recruits SIRT1 to regulate hepatic triglyceride (TG) accumulation through histone deacetylation. Hepatocyte-specific deletion of *Men1* resulted in increased susceptibility of liver steatosis in aging mice and high-fat diet-induced obese mice. Our findings demonstrate that menin is critical for the regulation of lipid metabolism in the liver.

Materials and methods

Animal experiments

Men1$^{flox/flox}$ mice [17] were bred with mice expressing the Cre recombinase driven by the albumin promoter (Alb-Cre, Jackson Laboratory). C57BL/6J mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (SLAC, CAS). Heterozygous (*Men1*$^{f/+}$-*Cre*$^{+}$) mice were backcrossed with C57BL/6J mice for five times, and then crossed to generate hepatocyte-specific *Men1* knockout mice (*Men1*$^{f/f}$-*Cre*$^{+}$, LMKO) and their age-matched littermate controls (*Men1*$^{f/f}$-*Cre*$^{-}$). LMKO and control mice older than 8 weeks were fed with a standard chow diet or a high-fat diet (Research Diets) for 14 weeks. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Real-time reverse transcription-PCR (RT-PCR)

Quantitative RT-PCR was performed using a LightCycler 480 System (Roche Applied Science). See Supplementary data for detailed information.

Histological and biochemical analysis

Paraffin-embedded liver sections were stained with hematoxylin and eosin for morphological analysis. For the detection of neutral lipids accumulation, liver cryosections were stained using Oil Red O (Sigma). Serum and liver cholesterol, triglycerides were measured using commercially available kits (BioVision) following the manufacturer's instructions.

Glucose tolerance test and insulin tolerance test

Mice were fasted for 18 h before glucose tolerance test, and were fasted for 6 h before insulin tolerance test. Mice were injected intraperitoneally with either 2 g/kg glucose or 0.75 U/kg insulin (Regular Humulin, Eli Lilly and Company). Glucose measurements were taken up to 2 h post injection using One-Touch Ultra glucometers (LifeScan).

Adenovirus infection

The adenovirus expressing menin was constructed and packaged using pIRES2-EGFP vector and ViraPower™ Adenoviral Gateway™ Expression Kit (Invitrogen). Primary mouse hepatocytes were infected with adenovirus expressing GFP and menin for 48 h in culture media. Tail vein injection of GFP or menin adenovirus in mice was performed for *in vivo* studies.

Cell culture

Primary hepatocytes were isolated from control or LMKO mice using collagenase (Sigma) perfusion, seeded in Hepatocyte Medium (ScienCell Research Laboratories), and maintained in maintenance medium (Low glucose DMEM, 0.1% bovine serum albumin). HEK293T cells were cultured and transfected as described previously [22].

Western blot, coimmunoprecipitation, and ChIP analysis

Liver protein preparation, Western blots and immunoprecipitation were performed as described previously [22]. The following primary antibodies were used: anti-menin (Bethyl Laboratories), anti-Sirt1 (Cell signaling), anti-CD36 (R&D Systems). ChIP assays using primary hepatocytes were performed as described by Upstate Biotechnology with antibodies against menin, Sirt1, acetyl-Histone H3 or normal IgG. A triplicate PCR was performed for three independent experiments using DNA fragments and primer pairs specific for upstream genomic regions of *CD36*.

Statistical analysis

Significant differences were analyzed using Student's *t* test. Error bars in graphs represent standard deviations (SD). Differences were considered significant if $p < 0.05$.

Results

The expression of Men1 is reduced in livers of aging mice

To investigate the potential correlation of menin and metabolic homeostasis in the liver, we examined expression of *Men1* in mouse model studies. Interestingly, we detected reduced expression of menin in the livers of aging mice. Aging is a crucial factor that negatively impacts liver function and metabolism. Reduction of Sirt1 has been linked to liver disorder during aging [5]. Our data showed the expression of hepatic menin and Sirt1 was significantly decreased in the 12-month-old mice compared to 12-week-old mice (Fig. 1A and B). Moreover, protein levels of menin in the white fat and livers of diabetic *db/db* mice were also significantly reduced (data not shown). Recent reports from our lab and other groups have shown glucose and insulin control menin expression, which suggests that the metabolic status could be responsible for the expression level of hepatic menin. All together, these results suggested that menin could be functional in age-associated metabolic changes in the liver.

Hepatocyte-specific deletion of Men1 leads to liver steatosis during aging

To elucidate the physiological roles of menin in the liver, we generated liver-specific *Men1* knockout mice (LMKO) by crossing *Men1*$^{flox/flox}$ mice with albumin promoter-driven Cre (Alb-Cre) mice (Supplementary Fig. 1A–C). The young LMKO mice (12 weeks) showed a normal metabolic phenotype. However, the liver weight of LMKO mice was 10% more compared to controls (Supplementary Fig. 2). The development of metabolic