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Hepatic conditional knockout of ATF6 exacerbates liver metabolic damage by repressing autophage through MTOR pathway

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ABSTRACT

The liver is a central metabolic organ. Activating transcription factor 6 (ATF6) acts as an ER stress responsive gene and is reported to attenuate hepatic steatosis. Over expressing a dominant-negative form of ATF6 exacerbates glucose intolerance and insulin resistance. In the present study, we used the conditional knockout technique to specifically knockout ATF6 in the mouse liver. We used qPCR to detect the mRNA levels of related genes. Western blot analysis was used to evaluate protein levels. Flow cytometry assay showed the apoptosis status. Glucose tolerance tests and insulin tolerance tests were used to determine glucose and insulin sensitivity. The results showed that liver specific knockout of ATF6 exacerbated HFD-induced hepatic steatosis and glucose tolerance. Abolished ATF6 exacerbated gluconeogenic metabolism by MTOR mediated down regulation of autophage. In conclusion, these findings suggest that therapeutic strategies by supplementing ATF6 may be beneficial for the treatment of glucose intolerance as well as insulin resistance in the high fat induced liver metabolic damage condition.

1. Introduction

The liver is a central metabolic organ that regulates several key aspects of glucose and lipid metabolisms, including gluconeogenesis and fatty acid oxidation. The aberrant accumulation of fatty acids in liver can contribute to the progression of some diseases, including diabetes and hepatic steatosis. Hepatic steatosis is one of the most common metabolic diseases, which can progress to cirrhosis, and finally hepatocellular carcinoma [1,2].

The stacking of unfolded proteins in the endoplasmic reticulum induces ER stress, also known as unfolded protein response (UPR). The UPR plays an important role in metabolic diseases such as type 2 diabetes and steatosis [3]. Activating transcription factor 6 (ATF6) acts as a UPR transcriptional factor which regulates expression of ER-associated proteins such as GRP78, CHOP and XBP1 [4,5]. ATF6 decreases the unfolded proteins in ER to alleviate ER stress, or if this cannot be achieved, by initiating various physiological processes, including cell differentiation, autophage, and inflammation [6]. ATF6 inhibits gluconeogenesis by disrupting CREB-CRTC2 interaction [7]. ATF6's target XBP1 can inhibit gluconeogenesis in obesity [8]. Whole-body ATF6 knockout mice show exacerbated hepatic steatosis and glucose intolerance [9,10]. Recently, Chen et al. reveals that ATF6 Increases fatty acid oxidation to attenuate hepatic steatosis through PPARα [11]. However, the function of ATF6 in conditional liver knockout mice remains unclear and mechanisms of ATF6 contributing to gluconeogenesis and lipid metabolisms are still not fully understood.

In the present study we show that mice with conditional liver knockout of ATF6 are prone to liver metabolic damage. These mice are with low levels of autophage when fed with high fat diet (HFD). The inhibited autophage levels are regulated through MTOR pathway in the knockout mice.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice at 8–9 weeks of age were purchased from

Abbreviations: ATF6, activating transcription factor 6; UPR, unfolded protein response; GTT, glucose tolerance test; ITT, insulin tolerance test; RES, resveratrol; DEX, dexamethasone; FSK, forskolin; TG, triglyceride; TC, total cholesterol.

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the Model Animal Research Center of Nanjing University (Nanjing, China). B6(Cg)-Atf6tm1Hota/J mice (purchased from The Jackson Laboratory) with floxed alleles of ATF6 were crossed with mice that express Cre recombinase in the liver (B6/JNju-Albem1Cin(icre)/Nju, purchased from the Model Animal Research Center of Nanjing University) to generate liver specific Atf6 deficient mice 9 ($ATF6^{-/-}$), which expresses a Atf6 mutant protein in liver. Atf6 deficient mice bear a conditional deletion of Atf6 exon 8. All mice were housed and maintained on a 12 h light-dark cycle and on a regular unrestricted diet. The mice were fed with either a normal standard diet (SD. 9% fat) or high fat diet (HFD. 45% fat) ad libitum with free access to water. All animal experiments were conducted under protocols approved by the Animal Research Committee of the Affiliated Hospital of Qingdao University. The $ATF6^{-/-}$ mice received 200 mg/kg/day of resveratrol (RES, Sigma-Aldrich, USA) by oral gavage (ethanol-dissolved stock solution diluted in phosphate buffered saline), or 2.5 mg/kg/day mixed in HFD of WY14643 (Sigma-Aldrich).

2.2. Analytical procedures

Blood glucose values were determined using a glucometer (One Touch Ultra; LifeScan, Milpitas, CA, USA). Serum and liver concentrations of triglyceride and cholesterol were determined using an automated Monarch device (Affiliated Hospital of Qingdao University, Shandong, China).

2.3. Tolerance test

For glucose tolerance test (GTT), mice fasted for 16 h received an intraperitoneal injection of glucose (1 g/kg). For insulin tolerance test (ITT), mice fasted for 6 h received an intraperitoneal injection of human insulin (0.75 IU/kg). Blood glucose concentrations were measured from tail blood at the indicated times.

2.4. Primary cell culture and treatment

Primary mouse hepatocytes were isolated from livers of mice and cultured as reported [12]. Cells were cultured in serum free medium and treated with or without dexamethasone (DEX; 1 μmol/l; Sigma-Aldrich), forskolin (FSK; 10 μmol/l; Sigma-Aldrich) or RES (5 μg/ml) for 6 h prior to harvest for further analysis.

2.5. Quantitative PCR

Total RNA was isolated from cells or liver tissues using TRIzol (Invitrogen, USA). cDNA was synthesized in a 10 μl reaction volume using ReverTra Ace qPCR RT Master Mix (TOYOBO, Japan) following

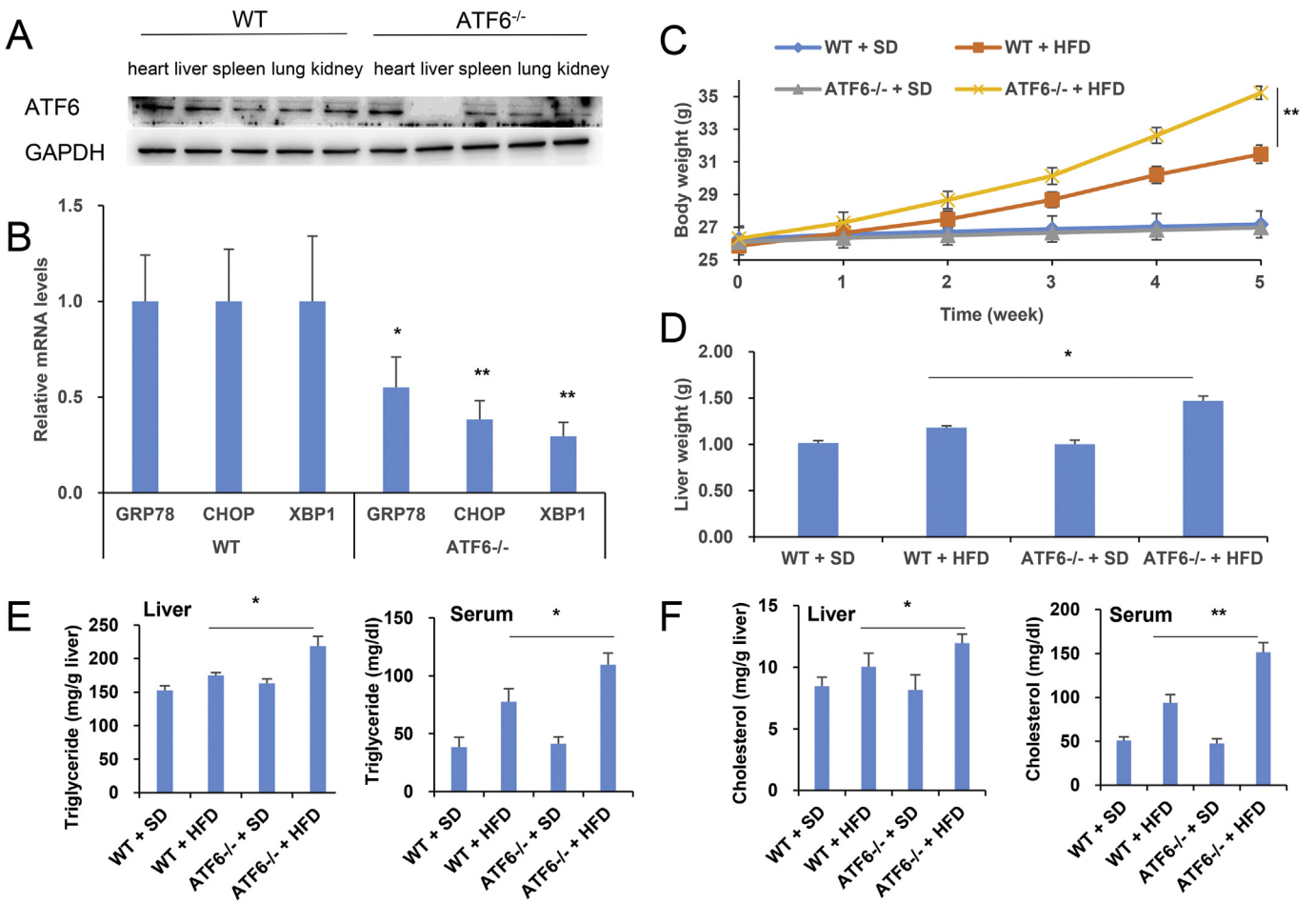

Fig. 1. Liver specific knockout of ATF6 exacerbates HFD-induced hepatic steatosis
(A)Western blot analysis of ATF6 expression in heart, liver, spleen, kidney and lung of WT and $ATF6^{-/-}$ mice. (B) qPCR analysis of GRP78, CHOP and XBP1 mRNA levels in liver tissues from WT (n = 6) and $ATF6^{-/-}$ mice (n = 6). (C) The body weight of WT (n = 6) and $ATF6^{-/-}$ mice (n = 6) on SD or HFD. (D) The liver weight of WT (n = 6) and $ATF6^{-/-}$ mice (n = 6) on SD or HFD. (E) Liver and serum triglyceride levels from WT (n = 6) and $ATF6^{-/-}$ mice (n = 6) on SD or HFD. (F) Liver and serum cholesterol levels from WT (n = 6) and $ATF6^{-/-}$ mice (n = 6) on SD or HFD. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01.

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