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Angiogenic factor with G patch and FHA domains 1 (Aggf1) promotes hepatic steatosis in mice

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ABSTRACT

Increased uptake of nutrients coupled with reduced activity leads to the development of a host of metabolic disorders in humans. In the present study we examined the role of angiogenic factor with G patch and FHA domains 1 (Aggf1) in the pathogenesis of steatosis, characterized by accumulation of lipids in the liver and consequently hepatic insulin resistance. We report here that Aggf1 expression was up-regulated in the liver in both genetically predisposed and diet-induced mouse model of steatosis. Aggf1 expression was also stimulated by free fatty acids in primary hepatocytes. Over-expression of Aggf1 in mice promoted steatosis. On the contrary, Aggf1 depletion ameliorated steatosis in mice. Mechanistically, Aggf1 activated the expression of gluconeogenesis gene and skewed the insulin signaling pathway to induce insulin resistance. Taken together, our data suggest that Aggf1 plays a role in steatosis *in vivo* and as such may be a new target in the development of therapeutics solutions against steatosis.

1. Introduction

The past decade has witnessed a peak in the incidence of metabolic diseases including dyslipidemia, hypertension, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) [1]. NAFLD represents a group of liver disorders as a result of excessive nutrition uptake (e.g., glucose, certain fatty acids) that range from simple steatosis to steatohepatitis to irreversible cirrhosis and hepatocellular carcinoma [2]. The pathogenesis of NAFLD encompasses a series of inter-connected pathophysiological events that include disruption of liver metabolic homeostasis [3], hepatic insulin resistance [4], infiltration of pro-inflammatory cells and augmented synthesis of pro-inflammatory mediators in the liver [5], accumulation of reactive oxygen species (ROS) in the liver [6], and accelerated transition of hepatic stellate cells to myofibroblast with enhanced fibrogenesis [7]. In the process of NAFLD development, these processes form an intertwined network that contains extensive self-amplifying feedbacks loops to collaboratively promote NAFLD progression. Many critical questions regarding the mechanisms underlying NFALD pathogenesis remain unanswered despite years of vigorous basic and clinic research.

Hepatocytes constitute the bulk of the liver parenchyma. When exposed to large quantities of excessive nutrients, hepatocytes undergo profound changes in terms of phenotype and function [8,9]. For instance, hepatocyte up-regulates its production of pro-inflammatory mediators including IL-1β, IL-6, IL-8, and TNF-α in the presence of high concentrations of palmitate [10]. Palmitate can also increase glucose levels in the liver by promoting gluconeogenesis in hepatocytes [11]. These changes are programmed by a host of proteins including both sequence-specific transcription factors/co-factors and signaling transducers [12,13].

Angiogenic factor with G patch and FHA domains 1 (Aggf1) was discovered as a pro-angiogenic protein in vascular endothelial cells in a genetic screen in patients with a rare congenital vascular disease [14]. Presently there is little evidence whether Aggf1 could play pathophysiologically relevant roles outside the vasculature. Our data as summarized in this report indicate that Aggf1 could

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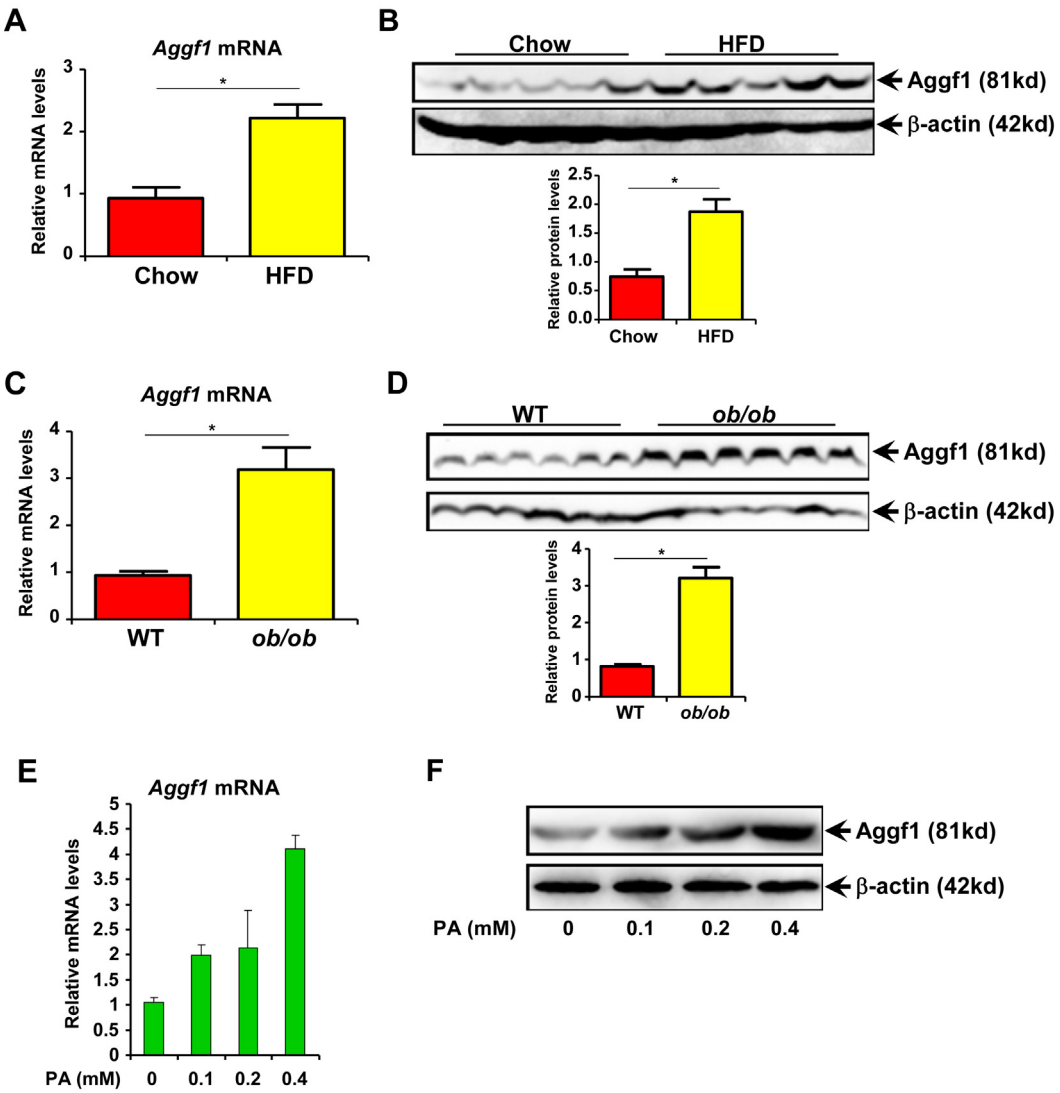

Fig. 1. ***Aggf1 expression is up-regulated in fatty livers in mice and in PA-treated hepatocytes***. (**A, B**) C57/BL6 mice were fed on an HFD or chow diet for 16 weeks. Hepatic Aggf1 levels were evaluated by qPCR (A) and Western (B). N = 5 mice for each group. (**C, D**) Hepatic Aggf1 levels were examined in 16-week old ob/ob mice and age-matched wild type mice by qPCR (C) and Western (D). N = 6 mice for each group. (**E, F**) Primary mouse hepatocytes were treated with or without palmitate (PA). Aggf1 expression was examined by qPCR (E) and Western (F). *, $p < 0.05$.

promote steatosis in mice in part by stimulating liver gluconeogenesis and suppressing hepatic insulin sensitivity. Therefore, targeting Aggf1 may be beneficial in the treatment of steatosis.

2. Methods and materials

2.1. Animals

All animal protocols were reviewed and approved by the intramural Committee on Ethical and Humane Treatment of Experimental Animals of Nanjing Medical University and in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. To induce steatosis, 6-8 week-old male *ob/ob* mice were fed on a chow diet for 4 weeks. Alternatively, 6-8 week-old male C57/BL6 mice were fed with a high-fat diet (D12331, Research Diets, New Jersey, USA) for 16 weeks. Glucose tolerance tests (GTT) and insulin tolerance test (ITT) were performed as previously described [15]. For GTT, mice fasted overnight were injected intraperitoneally with 2 g/kg glucose and blood samples were taken at the indicated intervals. For ITT, mice fasted overnight were injected intraperitoneally with 0.75 IU/kg soluble insulin. Blood glucose was measured using an Accu-Chek compact glucometer (Roche).

2.2. Cell culture, plasmids, transfection, and reporter assay

Primary hepatocytes were isolated from C57/BL6 mice as previously described [16]. Aggf1 promoter luciferase construct has been described previously [17]. Transient transfections were performed with Lipofectamine 2000 (Invitrogen). Luciferase activities were assayed 24–48 h after transfection using a luciferase reporter assay system (Promega). Experiments were routinely performed in triplicate wells and repeated three times.

2.3. Protein extraction and Western

Whole cell lysates were prepared as previously described [18]. Liver tissues were homogenized using the MagNA Lyser instrument (Roche) and re-suspended in RIPA buffer as previously described

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