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RESEARCH ARTICLES

Interstrain differences in the progression of nonalcoholic steatohepatitis to fibrosis in mice are associated with altered hepatic iron metabolism☆

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a major health problem worldwide. Currently, there is a lack of conclusive information to clarify the molecular events and mechanisms responsible for the progression of NAFLD to fibrosis and cirrhosis and, more importantly, for differences in interindividual disease severity. The aim of this study was to investigate a role of interindividual differences in iron metabolism among inbred mouse strains in the pathogenesis and severity of fibrosis in a model of NAFLD. Feeding male A/J, 129S1/SvImJ and WSB/EiJ mice a choline- and folate-deficient diet caused NAFLD-associated liver injury and iron metabolism abnormalities, especially in WSB/EiJ mice. NAFLD-associated fibrogenesis was correlated with a marked strain- and injury-dependent increase in the expression of iron metabolism genes, especially transferrin receptor (*Tfrc*), ferritin heavy chain (*Fth1*), and solute carrier family 40 (iron-regulated transporter), member 1 *(Slc40a1, Fpn1)* and their related proteins, and pronounced down-regulation of the iron regulatory protein 1 (IRP1), with the magnitude being A/J<129S1/SvImJ<WSB/EiJ. Mechanistically, down-regulation of IRP1 was linked to an increased expression of microRNAs miR-200a and miR-223, which was negatively correlated with IRP1. The results of this study demonstrate that the interstrain variability in the extent of fibrogenesis was associated with a strain-dependent deregulation of hepatic iron homeostasis.
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Keywords: NAFLD; Methyl-deficient diet; Liver fibrogenesis; Iron metabolism; IRP1; miR-200a

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease in the United States and other Western countries, refers to a broad spectrum of related hepatic disorders, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis [1–3]. Uncomplicated hepatic steatosis is generally considered a benign form of NAFLD and has a favorable outcome [2–4]; however, 10% of individuals diagnosed with simple steatosis will progress to NASH [5], and 30% of subjects with NASH will develop fibrosis and cirrhosis [4–6]. Currently, there is a lack of conclusive information to clarify the molecular events and underlying mechanisms responsible for this progression and, more importantly, for differences in interindividual NAFLD severity and sensitivity.

Abnormalities in several molecular processes, including altered hepatic lipid metabolism, oxidative and endoplasmic reticulum stress and inflammation, contribute to the NAFLD development and pathogenesis [7–10]. In addition to these alterations, recently accumulating evidence suggests that deregulated hepatic iron homeostasis may also be involved in the disease progression [11–13]. In our previous studies [14,15], we demonstrated that feeding mice a choline- and folate-deficient (CFD) diet for 12 weeks caused liver injury that resembled morphological features of NAFLD in humans; however, the magnitude of liver injury was strain-specific and varied among strains. This interstrain variability in severity of NASH-like liver damage was associated with a marked dysregulation of hepatic lipid and one-carbon metabolism, and the induction of oxidative and endoplasmic reticulum stress [14,15].

Several lines of evidence have indicated a tight interaction between lipid and one-carbon metabolism, the induction of oxidative and endoplasmic reticulum stress and hepatic iron homeostasis in the molecular pathogenesis of both alcoholic and nonalcoholic liver injury [16–19]. Importantly, it has been demonstrated that steatosis, inflammation and other NAFLD-related molecular abnormalities are attenuated as NAFLD progresses or after removal of the causative factor, while the peculiarities in iron-related metabolism are

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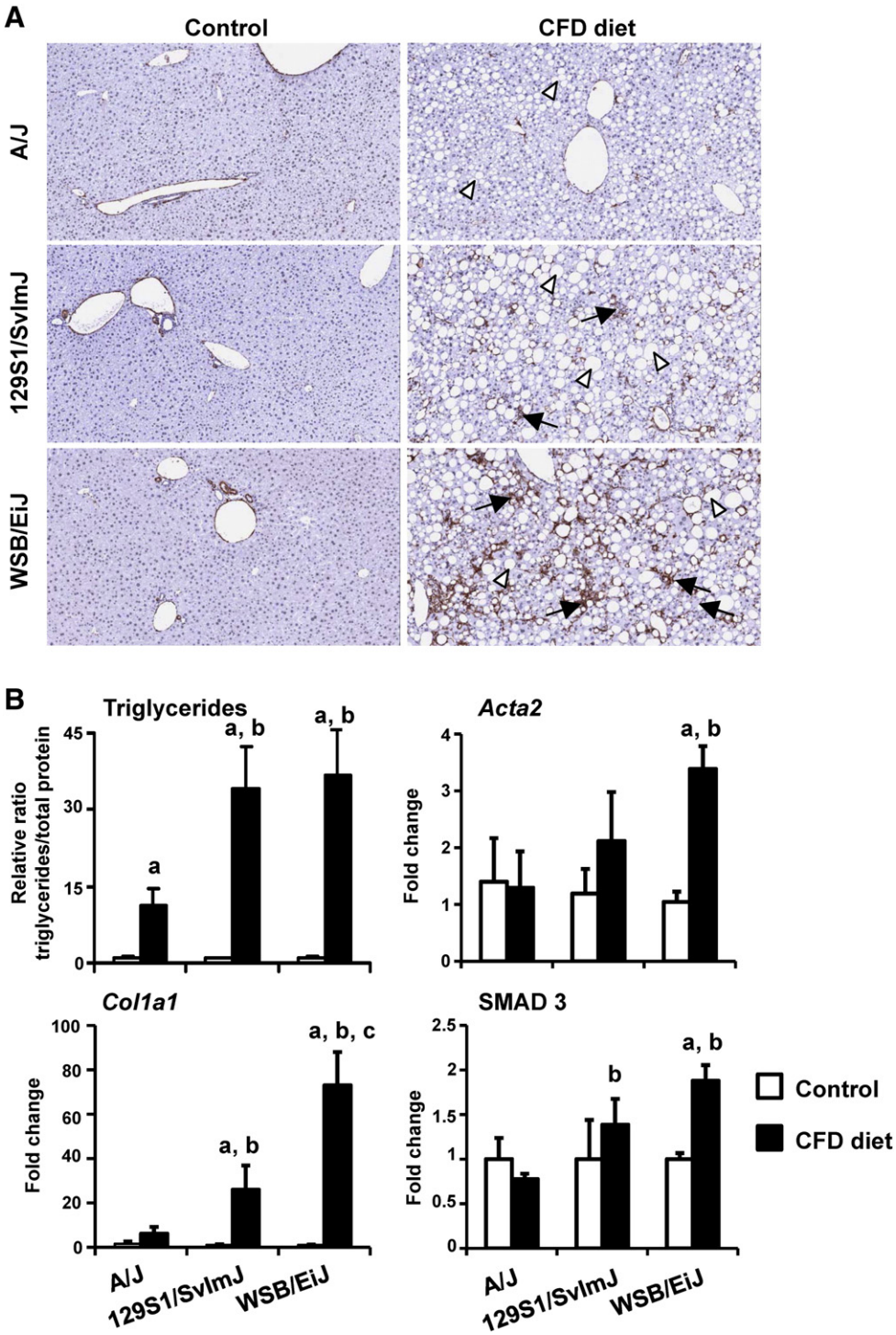

Fig. 1. NAFLD-associated liver injury in A/J, 129S1/SvImJ and WSB/EiJ mice fed CFD diet. (A) Steatosis (*open arrowheads*) and activation of fibrogenesis, evidenced by expression of α-SMA protein (*arrows*) in the livers of mice fed the CFD diet. Representative examples of control liver section and liver sections from mice fed the CFD diet are shown. (B) The level of triglycerides, expression of *Acta2* and *Col1a1* genes, and level of SMAD protein in the livers in A/J, 129S1/SvImJ and WSB/EiJ control mice and mice fed the CFD diet. [a]Significantly different from mice fed the control diet; [b]significantly different from A/J mice fed the CFD diet; [c]significantly different from 129S1/SvImJ mice fed the CFD diet (mean±S.D., $n=5$).

exacerbated [20,21]. This suggests that abnormalities in the hepatic iron metabolism may be a significant factor causing progression of NAFLD to more advanced disease stages; however, there is a lack of conclusive information of the underlying mechanisms linking iron metabolism alterations to the disease progression. Additionally, despite a well-established fact from human population studies showing that NAFLD in some individuals will progress to NASH, liver fibrosis and cirrhosis, but not in others, the determinants associated with this disease progression variability have not been fully investigated.