Deficiency of periostin protects mice against methionine-choline-deficient diet-induced non-alcoholic steatohepatitis

To the Editor:
Non-alcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease worldwide, encompasses a spectrum of diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) [1,2]. Periostin (encoded by POSTN), a matricellular protein, plays an important role in various inflammatory disorders, such as airway inflammation, skin inflammation, atherosclerosis and fibrosis [3,4], and actively contributes to tumour metastasis [5–7]. However, until the recent publication by Lu et al. [8], there was no known link between periostin and NAFLD. Lu et al. [8] found that hepatic periostin expression is dramatically increased in high-fat diet (HFD)-fed mice, ob/ob mice and db/db mice, as well as in NAFLD patients. Lu et al. [8] further demonstrated that periostin is a crucial contributing factor in aberrant hepatic triglyceride (TG) accumulation and in the pathogenesis of obesity-induced hepatosteatosis. However, the role of periostin in the pathogenesis of NASH remains unknown [9]. As an aggressive form of fatty liver disease, NASH is characterized by histopathological features, such as steatosis, inflammation and fibrosis, and is often accompanied by the metabolic syndrome, such as diabetes, insulin resistance and obesity. Moreover, some individuals with NASH eventually advance to liver cirrhosis and/or hepatocellular carcinoma, whereas hepatic steatosis usually has no serious clinical consequences [2,9]. Because genetically induced rodent obesity or HFD-induced hepatic steatosis does not progress to steatohepatitis, we now present data to determine the role of periostin in the development of methionine-choline-deficient (MCD) diet-induced NASH in mice.

To reveal the role of periostin in the development of NASH, we fed two groups of C57BL/6j mice with either regular chow or the MCD diet for 4 weeks. MCD diet-fed mice developed hepatic steatosis whereas chow-fed mice did not develop steatosis. The MCD diet also resulted in inflammation and fibrosis in mouse liver tissue (data not shown). We found that periostin was markedly upregulated and mainly distributed around steatotic hepatocytes in the MCD diet-fed mice by immunohistochemical staining (Fig. 1A). Western blotting and qRT-PCR analyses further demonstrated that the protein and mRNA levels of liver periostin were dramatically increased in MCD diet-fed mice compared with control mice (Fig. 1B and C). Moreover, we found that mice fed the MCD diet for 8 weeks, also developed NASH and showed phenotypes of steatosis, inflammation and fibrosis. The protein and mRNA levels of liver periostin in mice fed the MCD diet for 8 weeks were also significantly upregulated (data not shown). These data suggest that periostin may be involved in the development of MCD diet-induced NASH.

To determine whether periostin deficiency abrogates the progression of NASH, we used a periostin-deficient mouse model. Heterozygous B6;129-Postn<sup>tm1Jmol</sup>/J (Postn<sup>a</sup>) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). Periostin-deficient and periostin wild type mice were generated from crossing Postn<sup>a</sup> with Postn<sup>c0/c0</sup> mice. After feeding the MCD diet for 4 weeks, periostin-deficient mice exhibited significantly less TGs in their livers compared to the wild type group (Fig. 1D). The serum level of alanine aminotransferase was markedly decreased in Postn<sup>a</sup> mice compared to wild type mice (Fig. 1E), indicating that the MCD diet produced a more severe liver injury in wild type mice than in Postn<sup>a</sup> mice. Moreover, MCD diet feeding resulted in an increased collagen deposition in wild type mice, as shown by Sirius red staining; however, collagen deposition was significantly lower in Postn<sup>a</sup> mice than in wild type mice after being on the MCD diet (Fig. 1F and G), suggesting that Postn<sup>a</sup> mice exhibited significantly less histological fibrosis compared to the wild type group. We also found that mRNA levels of the inflammatory and fibrotic factors IL-6, TGF-β1, and α-SMA were markedly increased in livers of MCD diet-fed wild type mice compared to the chow diet-fed wild type controls; however, the mRNA levels of IL-6, TGF-β1, and α-SMA were significantly lower in the liver tissue of Postn<sup>a</sup> mice than in wild type mice after being on the MCD diet for 4 weeks (Fig. 1H). Therefore, these data suggest that a deficiency in periostin abrogates the development of MCD diet-induced NASH in mice.

In conclusion, our work demonstrates that periostin is highly expressed in MCD diet-induced NASH and that periostin knockout mice show a markedly lower degree of steatosis, inflammation and fibrosis compared with wild type mice after being fed the MCD diet. As previously mentioned, hepatic periostin levels are significantly upregulated in monogenic-induced obese mice and HFD-fed mice, as well as in patients with fatty liver disease [8]. Therefore, these data demonstrate that periostin is a potential diagnostic marker and therapeutic target for hepatosteatosis, NASH and even other liver diseases.
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Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

References


Fig. 1. Periostin contributes to the development of MCD diet-induced NASH in mice. The protein and mRNA levels of periostin in the livers of mice fed with either chow (n = 5) or MCD (n = 4) diets for 4 weeks were determined by immunohistochemical staining (A), Western blotting (B) or qRT-PCR (C) analyses. (D) TG contents were detected in livers of wild type and Postn−/− mice, fed with chow or MCD diet for 4 weeks (n = 4 in each group). (E) Serum alanine aminotransferase (ALT) levels were determined in wild type and Postn−/− mice, fed with chow or MCD diet for 4 weeks (n = 4 in each group). (F) Collagen depositions in the livers of wild type and Postn−/− mice, fed with chow or MCD diet for 4 weeks were detected by Sirius red staining. (G) Collagen deposition of the Sirius red stained positive areas in (F) were quantified by Image Proplus 6.0 (n = 4 in each group). (H) The mRNA levels of IL-6, TGF-β1, and α-SMA in the livers of wild type and Postn−/− mice, fed with chow or MCD diet for 4 weeks, were determined by qRT-PCR analysis. (n = 4 in each group. *p <0.05; **p<0.01; n.s., not significant).
TERT promoter mutation during development of hepatoblastoma to hepatocellular carcinoma

To the Editor:

We read with great interest the article by Eichenmüller et al. [1], who used whole-exome sequencing to describe the genomic landscape of hepatoblastoma (HB) cells and their progenies with hepatocellular carcinoma (HCC)-like features. They concluded that mutation of CTNNB1 and activation of the NFE2L2-KEAP1 pathway are important features in HB-development and further define loss of genomic stability and TERT promoter mutations as prominent characteristics of aggressive HB with HCC features. Although we appreciate these impressive findings, there are some important considerations that need to be stressed.

First, HB and HCC share a common CTNNB1 mutation, which leads to activation of Wnt signalling and the genesis of liver cancer in both adults and children. The oncogene TERT has been shown to regulate CTNNB1 expression, while conversely β-catenin can bind to the TERT promoter to positively influence TERT expression [2,3]. TERT promoter mutations are commonly involved in the last step of malignant transformation in association with CTNNB1 mutations in HCC [4]. Eichenmüller et al. showed that the expression of TERT was upregulated in both HB and transitional liver cell tumours (TLCT). However, TERT promoter mutations were identified in two thirds of TLCTs analysed but not in any instances of HB. They concluded that the TERT promoter mutation is a selective phenomenon for advanced HB with HCC-like features. However, only three patients were included as TLCT controls, and the cause(s) of their disease were not specified. Moreover, one of the TLCT controls did not possess mutations of the TERT promoter. This limited incidence of TLCT may influence the conclusion that TLCT is a genetically derailed progeny of HB. Analysis of TERT promoter mutations in paediatric HCC may assist in making such a conclusion more convincing.

Second, it has been recently reported that the TERT promoter mutation is an early event in liver carcinogenesis of cirrhosis, based upon analysis of the sequence of lesions from cirrhosis to low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), early HCC (eHCC) and small and progressed HCC. TERT promoter mutations occurred in a small proportion of dysplastic nodules (6% of LGDN and 19% of HGDN), and the prevalence increased with the degree of dysplasia. The prevalence of TERT promoter mutations was dramatically increased in eHCC (61%), suggesting a molecular shift had occurred following development of eHCC [5]. These findings suggest that TERT promoter mutations might be an important mechanism to drive malignant liver cells towards a more aggressive eHCC-like phenotype. TERT promoter mutations may therefore be a selective phenomenon of advanced HB with eHCC-like features. Furthermore, TERT promoter mutations were significantly associated with histological (pseudoglandular formation, cytological atypias, and unpaired arteries) and immunohistochemical (positive staining for GPC3 and HSP70) features of malignancy [5].

Therefore, Eichenmüller et al. should further evaluate their data by examining paediatric HCC patient samples for the aforementioned histological and immunohistochemical features. Alternatively, a larger case-control study that incorporates the above-mentioned points is required.

Conflict of interest

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References


Yuan Li1, Shasha Wu1, Shanshan Xiong1, Gaoliang Ouyang1*
State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Biology, School of Life Sciences, University of Puerto Rico at Mayaguez, Mayaguez, PR, USA

*Corresponding author. Tel.: +86 5922186091; fax: +86 5922181015.
E-mail address: oygldz@xmu.edu.cn

† These authors contributed equally to this work.

Shanshan Xiong
State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Biology, School of Life Sciences, University of Puerto Rico at Mayaguez, Mayaguez, PR, USA

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