literature alone. In addition, it is advised to perform studies on mild and severe mouse models of I/R injury and to validate results generated with these models on clinical material.

Conflict of interest

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.12.014.

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Hepatic hepcidin expression is decreased in cirrhosis and HCC

To the Editor:

Recent evidence published in this *Journal* showed the protective role of the iron homeostasis regulator hepcidin (*Hamp*) in iron overload-related liver diseases [1]. The study by Lunova *et al.* elegantly demonstrated that the knockdown of hepcidin promotes hepatic inflammation and fibrogenesis after feeding mice an iron-rich diet [1].

It is well known that perturbations of the iron metabolism, as it is the case in hemochromatosis, can lead to hepatocellular carcinoma (HCC). HCC represents the second most common cancer-related death worldwide and displays also the end-stage of liver diseases related to chronic viral or non-viral hepatitis.

As hepcidin deficient mice were more prone to develop fibrosis [1], which is itself a risk factor for HCC, deregulation of *Hamp* might also play a role in the progression of chronic liver disease to HCC development. Also alcohol intake, another risk factor for HCC development, lowers hepatic *Hamp* expression in a murine model of alcoholic steatohepatitis [2].

Regarding HCC, low *Hamp* levels have been reported in late stage murine and rat tumors [3,4]. As this downregulation might display a late, secondary, rather than an initial effect of carcinogenesis, we aimed at deciphering whether *Hamp* expression is already decreased in early hepatocarcinogenesis. We observed that mice treated with the carcinogen diethylnitrosamine (DEN), to induce hepatocarcinogenesis, showed decreased hepatic *Hamp* expression already in an early stage of tumor development (Fig. 1A). *Hamp* expression was also reduced in tumor tissues, compared to matched adjacent normal liver tissues, in a later stage of murine tumorigenesis (Fig. 1B).

To test the relevance of the observed decreased hepcidin in rodent HCC for human disease, we analyzed a large human Gene Omnibus (GEO) dataset (GSE14520 [5]), mostly consisting of hepatitis B virus (HBV)-related HCC samples. *Hamp* expression was strongly decreased in the majority of tumors compared to normal liver samples (Fig. 1C). This is in line with results from a small HCC cohort with mixed etiology [6]. Interestingly, serum hepcidin levels were shown to be decreased in patients with chronic hepatitis C [7]. To test for hepatic hepcidin expression in cirrhosis, we analysed two additional datasets containing cirrhotic liver samples. Cirrhotic tissues showed lower *Hamp* expression compared to healthy liver samples in an HBV-related cohort (Fig. 1D) as well as in HCV-infected patients (Fig. 1E).

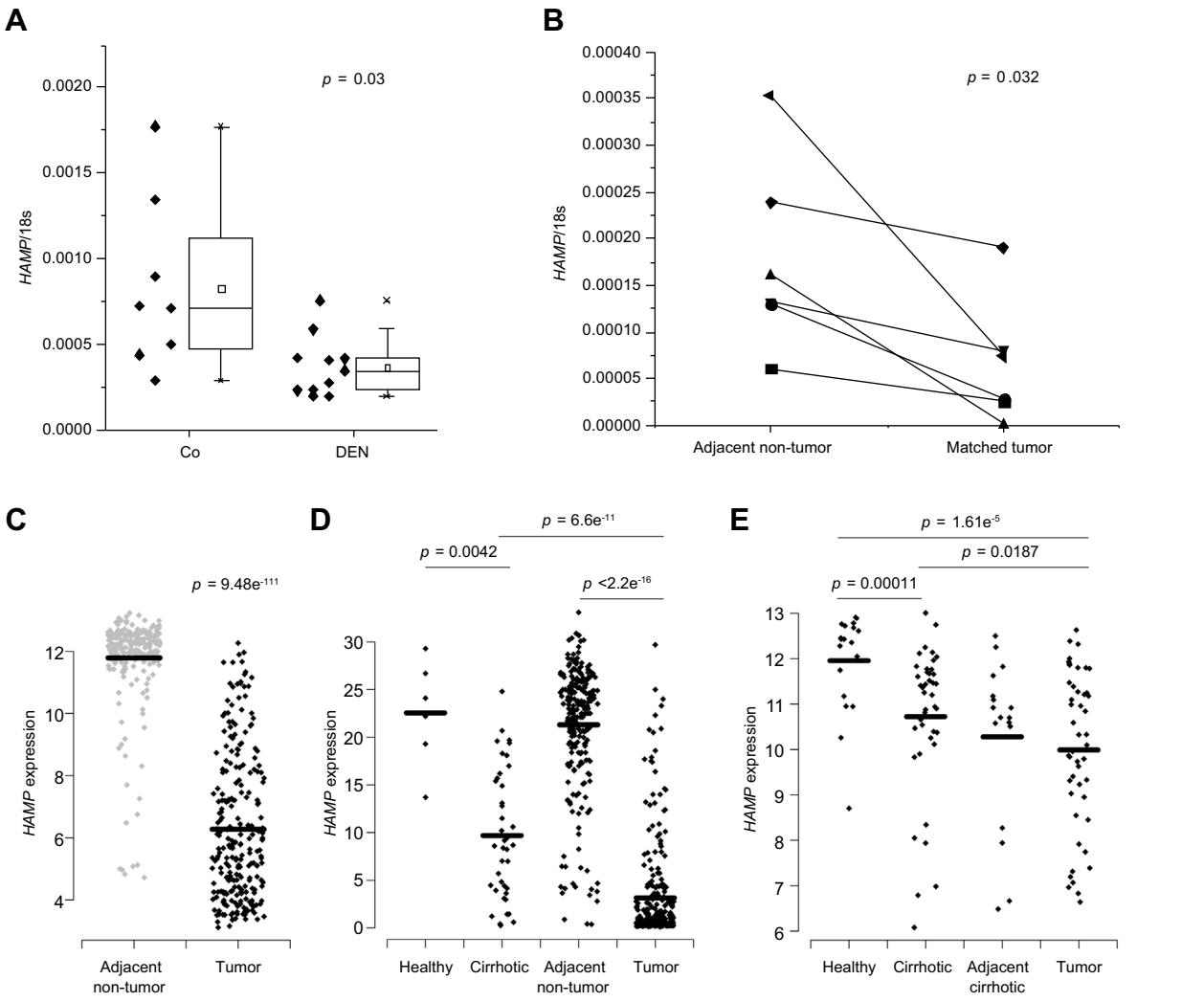

Fig. 1. *Hamp* levels in murine and human HCC. (A) Hepatic *Hamp* expression in non-tumorous murine liver tissue, 6 months after intraperitoneal injection of 5 mg/kg BW diethylnitrosamine (DEN) at the age of 2 weeks, compared to untreated control (co). Data are presented as individual values and box plots with median (—) and mean (□) of untreated control (co, n = 8) and DEN-treated (DEN, n = 11) animals. (B) *Hamp* expression in adjacent non-tumorous murine liver tissues and matched tumor tissues (n = 6), 8 months after DEN injection as described in (A). *Hamp* expression was normalised to *18s* expression (A and B). (C–E) Gene expression of *Hamp* in human datasets GSE14520 (adjacent non-tumor samples n = 247, tumor samples n = 239) (C), GSE25097 (healthy samples n = 6, cirrhotic samples n = 40, adjacent non-tumor samples 243, tumor samples n = 268) (D), and GSE14323 (healthy samples n = 19, cirrhotic samples n = 41, adjacent cirrhotic non-tumor samples n = 17, tumor samples n = 47) (E), downloaded from Gene Omnibus (GEO) and normalised using $\log_2$-RMA. Data are shown as individual values of expression. The statistical significance was determined by Mann-Whitney U test (A), paired sample *t* test (B), subsequent to confirmation of normal distribution, or Kolmogorov-Smirnov test (C–E).

Furthermore, *Hamp* mRNA levels were even lower in tumor tissue (Fig. 1D and E). Interestingly, hepatitis C virus (HCV) has been described to suppress hepcidin expression *via* generation of reactive oxygen species [8]. With HBV also inducing oxidant stress, this might also be true for HBV. Furthermore, *Hamp* expression can be transcriptionally activated by the tumor suppressor p53 [9]. As p53 is frequently suppressed in HCC [10], downregulation of hepcidin might be linked to p53 suppression.

In conclusion, these findings in the DEN mouse model and three human HCC cohorts strongly support a role of hepcidin deficiency not only as a model for iron-related liver disease, but also for other liver diseases leading to HCC. Therefore, hepcidin knockout mice presented by Lunova and colleagues [1] might be an interesting model to study progression of various liver diseases towards HCC.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Hepatic hepcidin expression is decreased in cirrhosis and HCC"

Hepcidin in chronic liver disease and hepatocellular carcinoma: The plot thickens

To the Editor:

We read with interest the letter by Kessler *et al.* [1] that demonstrated decreased hepcidin levels in various stress situations as well as in hepatocellular carcinoma. Lowered hepcidin levels seen in diethylnitrosamine-treated mice nicely complement earlier data on mice subjected to thioacetamide-induced liver fibrosis or administered Lieber-DeCarli diet (an experimental model of alcoholic liver disease), and suggest that diminished hepcidin levels represent a common reaction to various liver stresses [1–3]. Similarly, observations predominantly made in hepatitis B-infected patients extend previous findings, obtained in subjects with chronic liver disease due to hepatitis C infection or excessive alcohol intake, which all display reduced liver hepcidin levels [1,4]. Most importantly, in an elegant molecular analysis, Kessler *et al.* also showed that hepatocellular carcinomas exhibit diminished hepcidin expression, that was previously reported only in smaller studies.

Since hepcidin represents the central negative regulator of iron metabolism [4], these data reinforce the hypothesis that chronic liver disorders may promote development of acquired iron overload again triggering the progression of liver fibrosis and/or development of hepatocellular carcinoma. As an underlying mechanism, iron overload has multiple deleterious downstream effects, such as formation of reactive oxygen species, mitochondrial or lysosomal injury [5,6]. However, while several reports demonstrated an association between increased hepatic iron load and progression of liver fibrosis and/or HCC development [7], no such studies are available for hepcidin. This is rather surprising given the availability of multiple assays that can conveniently assess hepcidin serum levels. However, the extent to which these assessments can accurately reflect liver hepcidin expression or iron content in complex clinical settings, comprising multiple confounding factors, remains to be clarified. Nevertheless and without any doubt, such studies are of obvious medical and biological interest, although their interpretation will likely be complicated.

In that respect, the regulation of hepcidin production is complex and is affected, not only by iron metabolism, but also by various hepatic factors; by inflammation, erythropoietic drive, hypoxia etc. [4]. Moreover, hepcidin has a short half-life and displays a circadian rhythm [8]. Even more challenging will be to dissect whether the altered hepcidin levels represent a cause or consequence of liver disease/fibrosis progression. Finally, the deleterious effects of iron metabolism in liver carcinogenesis are further modulated by complex genetic factors at both the constitutional and functional levels; indeed, multiple genetic traits seem to impact hepatic iron content in patients with chronic liver disease [9]. While in-depth transcriptomic analyses reveal that expression of the *HAMP* gene could participate in a refinement of molecular classification of HCCs [10].

In conclusion, although the road ahead will likely be bumpy, it will be challenging to uncover the complex interaction between liver disease and iron metabolism. These interactions are now more important than ever given the rapid emergence of hepcidin-targeted therapeutic strategies that could be further modulated by host- and/or tumour-related genetic factors in the setting of personalized medicine.

Conflict of interest

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