Reviewer’s report

Title: Mouse model of carbon tetrachloride induced liver fibrosis: Histopathological changes and expression of CD133 and epidermal growth factor

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Reviewer: helene gilgenkrantz

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Major points

1. Histological data have to be improved:
   - Why do the authors claim that there is no cirrhosis? It seems at least, although it should have been better seen on Sirius red Staining that there is bridging fibrosis. More data have to be presented to confirm this point. Moreover, PCNA or Ki67 should be included to demonstrate the presence or absence of regenerative nodules. These results will allow to correlate EGF/EGFR expression to hepatocyte proliferation.
   - Hepatocellular carcinoma is not well characterized. Higher power field as well as a macroscopic view should also be included.

2. To explain why they found a downregulation of EGFR and EGF during the progression of fibrosis in mice, while some previous papers had shown an upregulation of EGFR in cirrhotic rats and in human cirrhosis, the authors suggest species differences. This conclusion is not convincing:
   Indeed, in a DEN-induced fibrosis/cirrhosis/CHC sequence in rat, Schiffer et al have found exactly the same results, namely:
   - a down regulation of EGFR protein and mRNA
   - a down regulation of EGF
   - an upregulation of TGFa
   The authors have to reinterpret their results with this view since equivalent results in a rat model have been obtained (HEPATOLOGY 2005;41:307).
   Moreover, to confort these data, a western blot showing the active phosphorylated form of EGFR should be given.

3. Discussion: “EGFR may possibly participate in the development of liver cirrhosis through a previously unrecognized mechanism” is not true: amphiregulin has been shown to participate in this process ( Perugonia et al Hepatol 2008; 48: 1251). It should be interesting to show the expression of other EGFR ligands such as amphiregulin or HB-EGF in their model.

4. The authors claim that CD133+ HSC are recruited during chronic liver injury, while only a correlation between up-regulation of different transcripts is shown. To confort this point and the fact that CD133 is downregulated in non-cancerous
tissue after Ccl4 withdrawal and not in HCC, an immunohistochemistry for CD133 should be included. Finally, there is no experimental evidence that CD133 HSC have contributed neither to liver regeneration nor to tumor progression (last sentence of the abstract) as it is claimed.

Minor essential revisions

5- Material and Methods: the authors should indicate when mice have been killed regarding the last oral CCl4 administration.

6- Discussion: the authors reviewed nine reports of cirrhosis induced by Ccl4 in mice. Their conclusion about this study is unclear. Moreover, in these papers, either HCC develop on a cirrhotic liver, or cirrhosis is incomplete and the animals do not develop HCC. How do the authors explain that in their hands, mice develop HCC without cirrhosis?

7- Last paragraph in the Results Section: Since all cell lines tested are hepatocytes or hepatoma cells, the authors wanted to determine whether Ccl4 promotes CD133 expression in existing PARENCHYMAL cells. Moreover, the authors should indicate whether there is a differential expression between non-tumorigenic and cancer cell lines of the same species.

8- The authors should indicate that CD133 up-regulation has also been demonstrated in human fibrosis, in the ductular reaction of chronically damaged human livers (Tsuchiya et al, Hepatol Res 2009; 39: 1080). They also have to discuss the different observations of CD133-positive and CD133-negative hepatocellular carcinoma in humans (BMC cancer 2009; 9: 324/ Ma et al, gastroenterol 2007/ Yin et al Int J Cancer 2007...)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'