Reviewer’s report

Title: Global analysis of DNA methylation in early-stage liver fibrosis

Version: 1 Date: 27 October 2011

Reviewer: A Tannapfel

Reviewer’s report:

Global analysis of DNA methylation in early-stage liver fibrosis In this study, the authors investigated genome wide DNA methylation profile during onset of liver fibrosis in a rat model. Towards this, the authors generated carbon tetrachloride (CCl4)-induced hepatic fibrosis in rats and performed a combination of methyl-binding protein (MBP) based precipitation (MBP-IP) and high-throughput DNA sequencing (MBP-seq). Subsequent bioinformatic analysis of their data revealed Spp1/osteopontin to be hypomethylated during hepatic fibrosis. Our review comments/recommendations on the manuscript of Komatsu et al are enumerated below:

I. Major compulsory revisions

Osteopontin has been reported to be upregulated in mice and rats by CCl4 treatment using immunohistochemistry and in situ hybridization by Lorena D et al. J Hepatol. 2006 (PMID: 16221502). Therefore the hypomethylation observed by the authors is consistent with a model in which Spp1 may be over-expressed during CCl4-induced hepatic fibrosis via hypomethylation. However a precise and direct validation of demethylation induced re-expression of Spp1 is required.

a. 5-aza-2’deoxycytidine (5-dAza-C) treatment of BRL 3A cells or alternatively 5-dAza-C treatment of primary cultures of normal human or rat hepatocytes followed by investigation of Spp1 mRNA and protein expression should be performed.

b. Transfection of in-vitro-methylated and mock-methylated Spp1_promoter -LucP construct in AH-130 cells followed by reporter assays should be demonstrated.

II. Minor essential revisions

a. The authors should consider citing earlier as well as recently published reviews that summarize DNA methylation in hepatocellular carcinoma such as Tischoff I et al. World J Gastroenterol 2008 (PMID:18350605), Sceusi EL et al. HPB (Oxford). 2011 (PMID:21609368) and Herceg Z et al.

Mutat Res. 2011 (PMID:21514401), in the introduction or discussion section appropriately.

III. Discretionary revisions
Fig 2A and C collectively indicate that the global hypomethylation observed in CCl4-induced hepatic fibrosis is predominantly contributed by intergenic region and hence is suggestive of a putative role of hypomethylation of repetitive elements in the genome, during the onset of fibrosis. The authors may consider commenting on this feature obvious in their data under the discussion section.

This study is well conceived with defined understandable objectives; however the manuscript needs to incorporate the aforementioned recommendations to be considered suitable for the scientific standards of your journal. We therefore recommend that this manuscript is suitable for publication in your journal conditional to the mandatory fulfillment of the recommended revisions.