Reviewer's report

Title: Methylation profiling of twenty promoter-CpG islands, the genes of which may contribute to hepatocellular carcinogenesis in China

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Reviewer: Dr Jean-Pierre Issa

Level of interest: A paper whose findings are important to those with closely related research interests

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In this paper, Jian and colleagues use methylation-specific PCR to establish a methylation profile for 20 genes in 29 cases of HepB positive hepatocellular carcinoma from China. They report lack of methylation for 15 genes/loci, complete methylation (normal/tumor) for TIMP3, nearly complete methylation for RASSF1, a relatively low level of methylation for CDH13, and variable methylation for P16. In the latter case, methylation was associated with the presence of liver cirrhosis.

Technically, this study has some limitations. First, no positive controls are described. In MSP, the lack of amplification using M primers could mean either lack of methylation or poor PCR conditions. Thus, it is imperative to have positive controls for each gene for which no methylation was found. Second, the results for TIMP3 and RASSF1 in the figure are opposite to what is stated in the text. This may be due to figure mislabeling. Still, it would be highly unusual to find TIMP3 100% methylated in all normal tissues and tumors. A technical problem is likely here, and controls are needed (negative controls in this case). Third, it is important to note that the sensitivity of MSP is such that functional conclusions based on these results are difficult to reach, particularly in the absence of data on expression in primary tumors. Finally, the use of agarose gels is also suboptimal for MSP (it reduces sensitivity), and the total number of cases is relatively small.

Nevertheless, with the exception of TIMP3, the results are generally consistent with previous reports. Indeed, we have recently reported an association between P16 (and ER) methylation and cirrhosis, a result confirmed here. Discrepancies between methylation results in different papers are likely technical given the plethora of available methods, and the very subjective nature of some of them. Of note, however, we also have not found CDH1 (E-cadherin) methylation in hepatocellular carcinoma, in contrast to previous reports. Finally, the results on Caspase 8 are interesting, and most likely reflect the fact that the region examined is not a CpG island, and therefore follows
different dynamics than the other genes.

On the whole, notwhistanding technical caveats, these results will be useful to researchers interested in hepatocellular carcinoma and in DNA methylation.

**Competing interests:**

None declared.