Author’s response to reviews

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

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PDF covering letter
Dear Editor:

I am pleased with the overall rating of both significance and validity of our work made by both reviewers and would like to thank the reviewers for their encouragements as well as constructive criticisms.

The major criticisms are concerning the inherent inadequacies of the PCR based approaches that was chosen in this study. Many papers in this field have presented the data without any additional validation of the nature of the PCR products by sequencing or restriction digestion. In this context, it seems generally accepted that MSP assay in conjunction with sequencing analyses is the best among all the available techniques for the methylation profiling of any CPG islands in cells. In this paper, all the PCR products were checked for their identities by sequencing. Hence, there is a remote possibility for any of our data in this report are artificial.

However, it has been correctly pointed out by both reviewers that there is no easy way to attribute to negative PCR reaction to the PCR artifacts from the real fact. Reviewer I have specifically requested a control experiment to confirm our observation from the MSP analysis in conjunction of DNA sequencing that both p14ARF and p15 INK4a were unmethylated in all twenty nine hepatoma patient’s samples. No PCR reaction should be involved. Due to the short supplies of the patient’s sample, we have to use the hepatoma cell line DNA instead. To this end, we carried out the Southern analyses of the genomic DNA of three hepatoma cell lines, HepG2, Hep3B and HuH-7 that was digested with methylation sensitive restriction enzyme Hpa II (Fig. 2, in the revised version). The data from both approaches lead to the same conclusion that both the p14ARF and p15INK4b genes are not methylated in all three cell lines. Therefore, our conclusion regarding to the lack of methylation of these two genes in human hepatoma should be correct, and the MSP analyses in conjunction with DNA sequencing used in this paper should have been properly carried out.

Reviewer II has specifically criticized the data concerning the TIMP3 gene in the first draft manuscript. On this point, I have to ask for the forgiveness of the reviewer for the mistake that we made in the first draft. In fact that the TIMP3 gene is unmethylated in all the tissue samples tested, exactly as the Reviewer II had correctly pointed out. We have corrected this error in the revised version.

Reviewer II has stated: “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……………… Of note, however, we also have not found CDH1 (E-cadherin) methylation in hepatocellular carcinoma, in contrast to previous reports.” Therefore, all the data and conclusion presented in this paper should have already had the support from both published data and the unpublished one made by the Reviewer II.

Before we ask the editor to accept this paper to publish in BMC Cancer, we would like reiterate our sincere thanks to both reviewers for their encouraging and positive suggestions and comments.

With best wishes and looking forward to hearing from you.

Yours Sincerely

Jingde Zhu