Author's response to reviews

Title: Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver.

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Author's response to reviews:

To Peter Newmark
Editor-in-Chief, BMC Cancer

Dear Prof Newmark and Ira xe Puebla,
the issues re-raised by the referee Zhu and by the statistician have been carefully evaluated and the paper amended accordingly.

Specifically:

a) Referee Zhu J.
We now think to have eventually caught the points raised again by reviewer Zhou, that we believe to have addressed as follows:
1) The methylation status of CpG islands in the context of the studied sequences after MSP PCR is now detailed in Material and Methods (pages 5, lines 25-27), Results (pages 7, lines 7-11) and Figure and legend 2. To this aim and for sake of clarity an additional panel illustrating the distribution of CpG islands in the context of the analyzed sequence has been included in Figure 2 (top).
2) For sake of clarity the exact number of cases analyzed in the different settings and the overall number of tissue samples investigated in the different lesion categories are now further detailed in Figure and legend 3 and in Table 3.
3) From the last sentence of point 1 raised by the referee, we guess he likely misinterpreted the percentage of methylation index reported in Fig 3 as a prevalence of methylated cases determined by MSP. The methylation index reported in Fig 3 was obtained by Real Time MSP as detailed in Materials and Methods: it is a quantitative assay of methylated alleles in each individual category of lesions. For sake of clarity and in keeping with the suggestions of the statistician we have now modified Figure 3 by showing data from a different even if confirmative statistical analysis (ANOVA test).

b) Issues raised by the statistician
1) The criteria for sample collection are now detailed on page 4, line 3-6. We clearly obtained an informed consensus from all the patients.
2) The heterogeneity of etiology/pathology of samples was due to the unselection and consecutive retrieval of the cases. This is now detailed on page 4, line 3-6.
3) We agree with the statistician that a few categories of lesions (namely hepatocellular adenoma and focal nodular hyperplasia) are of low sample size. Indeed these two categories of lesions were not by themselves the focus of our investigation (that was conversely represented by cirrhosis-hepatocellular nodules, HCC and normal livers); rather they allowed us to get an insight into the heterogeneity of methylation of lesions arisen in non hepatitic normal liver.
4) As requested by the referee we have now performed a more appropriate statistical analysis (ANOVA test) to correctly compare the methylation index from 3 different groups of lesions in both hepatitis and non hepatitis liver (Figure 3a and 3b). The analysis equally resulted statistically significant. Results and the new statistical analysis are now reported in the Abstract (lines 15-16 and 18-20), Materials and Methods (page 7 lines 2-6) and Results (page 8 lines 18 and 29). An additional author (E. Morenghi, statistician) has been
added to the list of authors.

Included is the amended version of the paper that we strongly hope to be, this time, suitable for publication in the journal.

Sincerely,
Massimo Roncalli MD PhD