Reviewer's report

Title: Down-regulation of SFRP1 as a putative tumor suppressor gene can contribute to human hepatocellular carcinoma

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Reviewer: Reinhard Dammann

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General

In their manuscript 'Down-regulation of SFRP1 as a putative tumor suppressor gene can contribute to human hepatocellular carcinoma', Huang et al. have analyzed the inactivation of the SFRP1 gene in 120 primary HCCs and 100 matching non-cancerous livers. Moreover, they have investigated the effect of ectopic expression of SFRP1 in HCC cell lines with low levels of endogenous SFRP1. In their report, the authors demonstrate that SFRP1 is frequently downregulated in HCCs and that reexpression of SFRP1 inhibits growth of HCC.

In general, the paper is interesting and provides new data on the expression and function of SFRP1 in HCC carcinogenesis. However, the methylation data of the SFRP1 CpG island promoter in the primary tumors are weak. The authors should evaluate methylation status of more primary specimen and correlate hypermethylation with downregulation of SFRP1 in these cases. Otherwise the result that 'low frequency of LOH at the SFRP1 locus couldn’t be a crucial genetic event in HCCs' (page 18) is clearly an overstatement, since the authors have not analyzed distinct genetic alterations like microdeletions or substitutions of the SFRP1 gene.

The manuscript is not well prepared and several issues need to be addressed.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. It would helpful to reveal the promoter methylation of SFRP1 in several HCCs and to correlate methylation with downregulation.

2. The abstract is not very well written and especially the fist sentence in the result section is misleading 'SFRP1 was significantly down-regulated in 48% (58/120) and 30% (30/100) HCC specimens as compared to adjacent non-cancerous livers, respectively'. What did the authors compare? Moreover they should indicate the significance (P=xxx) rather than to write 'significantly' in the abstract.

3. The authors have scored staining of SFRP1 in HCCs samples by immuno-histiochemistry on a scale of 1+ to 3+. This scale needs to be explained in the method section.

4. It is not clear if differences in Figure 3B and 3D are significant.

5. How did the authors score the downregulation of SFRP1 by RT-PCR in primary HCCs compared to corresponding non-cancerous specimen? This is an important issue since they observed a significant decrease in frequency of downregulation of SFRP1 (48%) compared to a previous report (91.5%).

6. In the whole manuscript, English language should be intensively corrected.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Figure 1A: Are these different cases or examples of same case? Is helpful to indicate case number above each paired probe.

2. Figure 2B, 3B and 3D: OD450 in the Y-axis should be explained in M&M or figure legends.

3. In the figure legends, significance should be indicated rather than to write ‘P<0.05 was consider
significant’.

4. Figure 5A: Several samples were not explained in the M&M section: Sk-hep1, Huh-7 and PLC. Are the two adult and fetal samples identical and how were they obtained? The term ‘spot’ for a point in a graph is inappropriate.

5. Material and methods section is too long and some points need clarification: Why did the authors add an U (Uracil at the 3’ end of a MSP primer? What means an A in brackets in a primer sequence? How do the authors precipitate RNA with chloroform? How did they obtain the SFRP1 cDNA?

6. Length of all PCR products (RT-PCR, MSP) should be mentioned in M&M and figure legends.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

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