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: Raphael SAFFROY

Reviewer's report:

General
The authors have assessed the role of SFRP1, a Wingless-type signaling antagonist, as a putative tumor suppressor gene in hepatocellular carcinoma. SFRP1 mapped onto chromosome 8p12-p11.1 known as a frequent loss of heterozygosity region in human HCC and is a component of a signaling pathway known to play an important role in hepatocarcinogenesis. This gene have been previously reported to be down-regulated in HCC by transcriptomic approaches.

In the present study, the authors confirm the previously described role of promoter hypermethylation as an important event for the down-regulation of SFRP1 in HCC. They demonstrate with RNA interference and cell transfection technologies a role of this gene in cell proliferation. So it’s an interesting study confirming the suspected role of this gene in hepatocarcinogenesis. The study is clear and well defined. However, this study raises some comments, in particular concerning LOH and RT-PCR analysis:

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

1) ?beta-catenin, another protein involved in the Wnt pathway, have been described to be mutated in 20 to 40% of human HCCs and these mutations have been described to be more prevalent in HCCs related to HCV than HBV. In this study, authors indicate that no correlation was observed between HBV infection and down-regulation of SFRP1. However, no information are given concerning the viral status of the 120 patients. How many are HBV or not ? More generally, we need more clinical informations (age, gender, etiology) about the patients studied in “Materials, Tissue specimen” section.

2) Primers designed for beta-actin appears to not be absolutely specific. Indeed, non-specific amplification with alpha-actin is possible. Moreover, authors claim that to avoid DNA contamination, all primers were designed to span at least one exon. But to avoid genomic DNA contamination, primers must span at least one intron. And primers designed for SFRP1 and beta-actin span only one exon without intron. So they don’t avoid eventual DNA contamination. Authors must change their sentence and to specify if controls have been performed to confirm absence of influences of genomic DNA contamination and alpha-actin cDNA amplification in their results.

3) LOH analysis were performed using non-cancerous livers as reference. But the ideal reference for LOH analysis is a non pathologic tissue of the same patient because non-cancerous livers can also present LOH in particular if they are cirrhotic. So results can be under-estimated. Authors must specify if the tissues studied are cirrhotic or not.

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

Minor essential revisions:

1) In “LOH at the SFRP1 locus in HCCs” section and abstract, authors specify that 2 microsatellites were studied and LOH was found for 6 and 3 of the informative cases, where a total of 8 HCC specimens were considered as involving LOH. This sentence is not clear. Is it one case with the 2 microsatellites altered and 7 cases with only one ? If it is the case, these 7 patients have not a confirmed loss of one SFRP1 gene allele.

2) Always in this section, authors have performed LOH analysis in 46 pairs of HCC specimens. Why 46 ? Is it correspond to the informative specimens on the 2 microsatellites ? If not how many are informative ? If yes, authors must to clarify it.

3) Fig 4 shows 2 examples for LOH but the cases exhibited are not really informative because we have not a clear separation of the allelic bands, allowing objective interpretation of LOH. Interpretation is questionable between microsatellite instability or LOH.

4) Fig 6: we don’t understand the signification of the 8 lines of circles for each sample studied. Authors must to specify it.
5) In “DNA methylation of the SFRP1 promoter in primary HCCs” section, the term “quantitatively” is not appropriate.
6) Some writing mistake must to be corrected (page 22 interestedly-interestingly, page 18 expressed and not expression ...)

Discretionary Revisions (which the author can choose to ignore)
1) Significant SFRP1 expression was found in 4 cell lines. But DNA methylation status is specified only for
2. It would be interesting to give also the DNA methylation status of HuH-7 and MHCC-L cell line.
2) It would be also interesting to give the LOH status of the 2 HCC positive specimens studied for DNA
methylation (210C and 230C).

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

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:
I declare that I have no competing interests