Reviewer’s report

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

**Version:** 2  **Date:** 3 May 2007

**Reviewer:** Monica Anzola

**Reviewer’s report:**

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

In general, authors have responded to the major compulsory revisions previously suggested. However, there are still some points that are not clear and should be clarified.

1. Samples analysed. Semi-quantitative RT-PCR was carried out in 120 HCC samples (58 of them showed down-regulation of SFRP1). Then, real time RT-PCR was carried out in 46 informative cases (35 of them showed down-regulation of SFRP1). Which cases are these? Why were they selected? IHC was carried out in additional 100 pairs. Were these samples different from the HCC samples used for RT-PCR?

2. According to the text, authors evaluated the expression level of SFRP1 in available cell lines. In materials and methods they describe 15 liver tumour-derived cell lines (3 of them have been added from the previous manuscript, why?). But they only showed results for 8 of them in this experiment. Have they analysed all the cell lines for the expression level of SFRP1 or only those 8?

3. Author analysed exogenous SFRP1 expression. In this analysis they use YY-8103 cell line which previously showed not to express the gene, Hep3B (which is supposed not to expressed the gene, but it has not been included in previous results), and SMMC7721 (which previously showed not to express the gene). If there was weak or no expression of SFRP1 in Bel7402, SMMC7721, Bel7404 and YY8103 cell lines, why did they not do these experiments with these cell lines? It is not clear at all the use of one cell line or another along the manuscript.

4. DAC and TSA treatment was employed in 3 cell lines, why did they choose these ones? Because they are supposed not to express endogenetic SFRP1? How do they know? (Expression levels in QGY-7701 and MHCC-H have not been described previously in the manuscript).

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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)

1. English language should be intensively revised in the whole manuscript (not only the suggestions made by the reviewers)

2. The use of the term “respectively” is sometimes unnecessary or not clear. Page 2 (abstract) “SFRP1 was significantly……, respectively” (The term respectively is not necessary) and “The overexpression ….., respectively” (What does it mean? it is not clear what it refers to). Page 13 “…. an inhibitory of histone…, respectively (the meaning is not clear)

3. When describing LOH analysis size of PCR products should be included.

4. Table 2 has some mistakes, misprinting, and age data are not concordant.

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Discretionary Revisions (which the author can choose to ignore)

1. I still think that materials and methods are a bit long. Authors say that they have simplified the procedure of RNA extraction but it is just the same as the previous one (and it is supposed to be the normal Trizol protocol suggested by the manufacturer which can be followed reading the instructions provided by the manufacturer).

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:** 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