Reviewer’s report

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

Version: 1 Date: 16 February 2007

Reviewer: Monica Anzola

Reviewer’s report:

General
In general this manuscript seems to be coherent and scientifically sound. The manuscript represents a lot of work, a large number of patients, appears methodologically sound and provides interesting information.

1) General comments

Dr. Huang et al. have studied the status of SFRP1 gene in HCC patients from China. This gene has been described to be frequently inactivated by promoter methylation in many human cancers. Authors explored the contribution of SFRP1 down-regulation in hepatocarcinogenesis. They investigated the expression of the gene by RT-PCR and IHC, the overexpression and knockdown of SFRP1 by cell growth and colony formation and promoter hypermethylation by MSP or bisulfite-treated DNA sequencing assays. They also studied LOH at this region.

They found that SFRP1 was significantly down-regulated in HCC patients and that overexpression of the gene could significantly inhibit the cell growth and colony formation of several cell lines. RNA interference analysis showed that SFRP1 can promote cell growth in the cell line studied. LOH was found 13% of the informative cases and DNA methylation in 2/3 HCCs without SFRP1 expression.

In summary the authors suggest that the down-regulation of SFRP1 by genetic or epigenetic events could contribute to hepatocarcinogenesis.

2) Specific comments for revision

In general this study is quite straightforward, but some points should be revised.

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

- Clinico-pathological data should be better explained. A table with these data should be included or data should be included briefly in the text (a brief description of the tumours and patients). They should document clinical details because they pointed out that there are no relationship between the presence of some of the SFRP1 alterations studied and clinico-pathological data, so it would be helpful to know the characteristics of the sample.

- How many tumours have the authors analysed? These data are a bit confusing. RT-PCR was carried out in 120 HCCs and 100 adjacent tissues? Why? LOH analysis was carried out in 46 cases? Or only 46 of the cases were informative? If so, were the 46 cases informative for both markers? No tissue from individuals without any tumour has been used as control (normal liver)? Non-tumour tissues were adjacent to the cancerous nodules? Did any of them present any alteration?

- Authors should explain the limitations of their methods. In page 18 they describe “partially or complete unmethylated CpG islands” detected by MSP, how can they determine the extent of methylation with this method? Or was it detected by bisulfite-treated DNA sequencing assays?

- In general the discussion is a bit poor. A more completed review of several previous publications of SFRP1 should be included and discussed in correlation to their findings. For example, they found a lower frequency of SFRP1 down-regulation than a previous report. They should try to explain these differences. In general, they only comment the results but they do not discuss them or explain them.

- In the discussion, when the authors discuss about LOH presence they point out that they have analysed two microsatellite markers different from one previous report. Why did they select these two markers? How far are they from SFRP1 locus? How good are they to determine LOH at SFRP1 locus? They conclude that LOH is not a common event, but with only two markers this is difficult to assess. They do not make any comment about other findings described in other papers both for HCC or other tumour types.

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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)

- Which are the criteria for selecting in each analysis one or another HCC cell line among the 12 described
in materials and methods?
- There should be a clear listing of the major findings, samples analysed with each methodology, with all the numerators and denominators included and p-values given when possible.
- Although it is overall well written, editing for common English usage needs to be done. There are some grammatical and typing mistakes. For example: page 2 second paragraph “we”/”We”, page 3 line 1 “this cells”/”these cells”, page 4 line 2 “one of the most common cancer”/”one of the most common cancers”, page 14 line 9 “wasn’t”/”was not”, etc. These are some examples, but they should revise the whole text for grammar and misprinting mistakes.

Discretionary Revisions (which the author can choose to ignore)
- The methods are appropriate and well described, and there are sufficient details provided to replicate the work. However, in my opinion in some cases, such as RNA extraction, the methodology described is a bit long.
- In page 14, the paragraph about LOH “In general, our findings above implied…. “ should be included in the discussion.
- Is the D8SAC016868 marker well named or is it AC016868?

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