Author's response to reviews

Title: Secreted frizzled-related protein promotors are hypermethylated in cutaneous squamous carcinoma compared with normal epidermis

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Author's response to reviews: see over
Dear editor,

Thank you for giving us a chance to revise the manuscript, we also thank the reviewers for their good suggestions on our manuscript. We have revised the manuscript according to the comments of the two reviewers. The point to point responses are listed below.

**Editor's Request:**

1. Please revise the Methods section of your manuscript to include the names of the ethics committees that approved your study. We recommend the following format:

   "This study was approved by the ethics committee of [xxx] Hospital [or University]." If more than five ethics committees approved your study, you may wish to list the names of the committees in an additional file.

   **Response:** Thank you for your advice. I have included the names of the ethics committees that approved our study in the Methods section according your required format. Thank you.

2. Copyediting: We recommend that you copyedit the paper to improve the style of written English.

   **Response:** Thank you for your suggestion. To improve the style of written English of our manuscript, we revised the whole paper by Edanz (www.edanzediting.com/bmc1) before it was resubmitted to your magazine. These changes will not influence the content and framework of the paper.

   **Response:** We have added a table (Table 3) in the results section to demonstrate the methylation frequency comparison of *SFRP* CpG sites in three tissue groups in
our revised manuscript. Additionally, we also added some information about the study population in the first paragraph of the results section. Thank you.

**Reviewer #1**

1. The word “Data” requires a plural.

   **Response:** Thank you for reminding us about this. We have changed them according your comments in our revised manuscript.

2. The word “Spectra” requires a plural.

   **Response:** Thank you for your reminding. We have changed it in our revised manuscript.

3. Acronyms should be spelled out at their first use (e.g. ROC-line 94)

   **Response:** Thank you for your good advice. We have spelled out the ROC in our revised manuscript.

4. There should be a comma separating the independent clauses of a compound sentence, and a list of three or more items should have a comma between the last two items in the list.

   **Response:** Thank you very much for your raising this issue for us. We had made corrections in line with your comments when revising our manuscript. Thanks a lot.

5. The use of SFRP indicates that you are referring to the gene, so there is no need to write gene after it, otherwise refer to it as SFRP gene.

   **Response:** Thank you for pointing this out for us. We have made correspondence corrections according to your comments in our revised manuscript.
6. How was the histological classification obtained? Were pathology reports used for this?

**Response:** Thank you for your comment. The histological classification was obtained according to pathological features, and pathology reports were used for it.

7. Line 126- omits recently published work by Schiefer et al, 2014 re SFRP methylation status in glioma cell lines.

**Response:** Thank you for your good advice on our manuscript. We have added this study in our revised manuscript.

8. Line 175 states “may be one of the best biomarkers for melanoma diagnosis.” I assume this is a typographic error since the study only dealt with SCC.

**Response:** We are very sorry for our carelessness in our manuscript. Thank you for your reminding. We have made corrections according to your comment in our revised manuscript.

9. What do SFRP_1 and SFRP_2 refer to in Table 2?

**Response:** Thank you for your advice. The SFRP_1 and SFRP_2 refer to two amplified fragments of *SFRP* in Table 2. We have made annotation for them in our revised manuscript.

10. Why not SFRP3?

**Response:** Thank you for your advice. The reason why we did not choose SFRP3 was that SFRP1, SFRP2, SFRP4, and SFRP5 are present thought to play a key role in Wnt signaling pathways. Additionally, these genes are extensively studied in other human cancers. So we did not choose SFRP3 in our present study, and we will
do it in our future studies. Thank you.

In a word, we found the reviewer’s comments are quite helpful, and we have revised our paper point-by-point according to the good suggestions. Thank you the review again for his good advice.

**Reviewer #2**

**Major compulsory revision:**

1. Please change the title as followed or to something similar to: SFRP promotors are hypermethylated in cutaneous squamous carcinoma compared to normal epidermis.

   **Response:** Thank you very much for your kind suggestion. We have changed the title of my manuscript according to your advice in our revised manuscript. Thank you.

2. In background (line 51), the authors mentioned that there are no data on SFRP in cutaneous SCC. It looks that they have missed the paper by Pourreyron et al. (Plos One, 2012) where the authors showed dysregulation of SFRP1 and 2 gene expression in cutaneous SCC compared to normal skin. I suggest the authors to mention this study in the background section.

   **Response:** We are very sorry for our negligence in our manuscript. We have made correction according to your comment. In the revised paper, we have mentioned this study in the background section of our manuscript. Thank you for your reminding.
3. Could the authors please add the body sites of the tumours and normal skin, the immune status of the cancer patients, as well as the number of poorly/moderately/well-differentiated tumours in the material and methods section?

**Response:** Thank you for your advice. We have added the body sites of the tumours and normal skin samples in the material and methods section of our revised manuscript. We are very sorry to tell you that we did not test the immune status of the cancer patients, as well as the number of poorly/moderately/well-differentiated tumours in our present study. We will do them in our future study according to your comments. Thank you again for your good suggestions.

4. The authors suggest that SFRP1 promotor methylation could be used as a potential marker of cutaneous SCC early diagnosis (fig 2). I have two comments about these data. The first one is that I don’t find the ROC curve of SFRP1 CpG1_1 site very convincing and I would like to authors to calculate the area under the curve (AUROC) to determine the accuracy of the test. My second comment relates to the early diagnosis. Having not included any pre-cancerous skin lesions such as actinic keratosis in their study, I am not sure the authors can claim that SFRP1 promotor methylation could be used as a marker of early tumorigenesis. Could the authors please elaborate on this?

**Response:** Thank you for your comments. We have made some changes according to your advice when revising our manuscript. In this study, we in total analyzed 17
CpG island methylation of *SFRP1*. In the revised manuscript, we excluded the CpG sites with a *p*-value more than 0.05 and ROC less than 0.7. Finally, six CpG sites were left for analysis. We have added the area under the curve (AUROC) and *p*-value in our revised manuscript to determine the accuracy of the test (Fig 2).

Thank you for your advice.

**Answer to the second comment:** In the present study, we found that the *SFRP1* methylation frequency was significantly higher in the cutaneous SCC tissues than other three *SFRPs* (*SFRP2, SFRP4, and SFRP5*). We also observed a significant increase in the methylation rate of different *SFRP* CpG islands with higher pathological levels. Finally, we performed ROC curve analysis of *SFRP1* CpG island methylation, and it also showed some significant results. So we speculate that *SFRP1* promotor methylation could be a useful tumor biomarker for cutaneous SCC early diagnosis. However, restricted by some limitations of study, this speculation needed to be confirmed in future studies. Thank you for your comments on our manuscript again. We will continue to do what you mentioned in our future studies.

5. In the discussion, the authors should discuss their results with Pourreyron et al. paper as well as with the data provided by the Human Protein Atlas on cutaneous SCC ([www.proteinatlas.org](http://www.proteinatlas.org)).

**Response:** Thank you for your reminding. We have discussed the results of Pourreyron et al. paper and the data provided by the Human Protein Atlas on cutaneous in the discussion section of our revised manuscript. Thank you again.
Minor essential revision:

1. Having worked on tumours from Chinese patients for this study, it would be interesting to include Chinese statistics in the background section, if such numbers are available.

   Response: Thank you for your good advice. We have included Chinese statistics in the background section in our revised manuscript.

2. The transition between paragraph 1 and 2 in the background is quite poor. I would delete lines 35-37.

   Response: Thank you for your suggestion. And we have delete lines 35-37 in my revised manuscript.

3. Line 45: please change leads for leading and contribute for contributing.

   Response: Thank you for your reminding. We have changed leads for leading and contribute for contributing in our revised manuscript.

4. Line 59: please add department after dermatology

   Response: Thanks a lot for you reminding us about this. We have added department after dermatology in our revised manuscript.

5. Could you please comment on how the adjacent skin specimens were checked for absence of tumour material?

   Response: Thank you for your comment. According to references, tissue adjacent to carcinoma about 2 cm is adjacent tissue. We take the adjacent skin specimens based on this. Thank you.
6. Line 101: aberrant methylation was detected in each tissue group compared to what?

**Response:** Thank you for your advice. We have changed this sentence in our revised manuscript.

7. End of line 122 and 127: authors are requested to supplement some more references.

**Response:** Thank you very much for pointing this out for us. We have added some references on the basis of your comments in our revised manuscript.

8. Could you please comment on why you chose to study SFRP1 methylation as an SCC marker and not the other SFRP which according to your results are also hypermethylated in cancer?

**Response:** Thank you for your comment. The hypermethylation of SFRPs family have been studied extensively at present, especially *SFRP1*. So we choose *SFRP1* in our study, which has been already studied in cervical cancer. We will continue to complete the methylation markers of SFRP2, SFRP4 and SFRP5 in our future study. Thank you again for your advice.

9. I feel that SFRP immunostaining on the samples used for the present study would strongly improve the manuscript and confirm the results. Since good antibodies are available for SFRP immunostaining on paraffin sections, I would recommend the authors to perform such staining on the tissues sent to pathology.

**Response:** Thank you for your suggestions. We are very sorry for that we did not use *SFRP* immunostaining on the samples we used for the present study.
Considering your comments, we will do it in our future study to improve the quality and confirm the results of our study. Thank you for your suggestions again.

10. Line 175: am I right to think that the authors meant for cutaneous SCC diagnosis and not for melanoma diagnosis?

**Response:** I am very sorry for my carelessness. We have changed melanoma diagnosis for cutaneous SCC diagnosis in our revised manuscript.

11. Table 1: could you provide the age and mean age for both groups please (cases and controls)?

**Response:** Thank you for your advice. We have provided the age and mean age for both groups (cases and controls) in our revised manuscript. In addition, we have provided the $p$-value calculated for sex and age in our manuscript. Thanks a lot.

12. Table 3: could you please define N and H and mention how many tumours there were for each stage?

**Response:** Thank you very much for your suggestion. We have defined N and H and mentioned how many tumours there were for each stage in our revised manuscript.

13. Fig1A: isn’t it SFRP1 and not SFRP2?

**Response:** We are very sorry for our carelessness in our manuscript. And we have made corresponding changes in accordance with your comments in Fig1A. Thank you for pointing this out for us.
We acknowledge the editor’s and the reviewers’ comments very much, which are valuable in improving the quality of our manuscript.

Thank the reviewers’ comments on our manuscript again. We hope that the revised manuscript has addressed all the criticisms raised by the reviewers and that the manuscript is now suitable for publication in your journal.

Yours sincerely,

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