Author's response to reviews

Title: N-terminal and C-terminal Heparin-binding Domain Polypeptides Derived from Fibronectin Reduce Adhesion and Invasion of Liver Cancer Cells

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Author's response to reviews: see over
Dear Sabina Alam,

We have revised our manuscript (MS: 1719495825330849) entitled N-terminal and C-terminal Heparin-binding Domain Polypeptides Derived from Fibronectin Recuce Adhesion and Invasion of Liver Cancer Cells. We highlighted all word and sentence changes in red colour.

Following is a point-by-point commentary explaining how each of the reviewer’s comments has been addressed. The reviewer comments are in italic and our replies are in blue.

Reviewer, Orlando Musso:

Major Compulsory Revisions

1. Page 19, Discussion, 2nd paragraph: “The present study demonstrated (...) had the ability to prevent lung metastasis of MHCCC97H cells...” This is an over interpretation of the results. The reduction in the number of metastatic foci is mild (~30%). No experimental data have been presented indicating that these polypeptides can prevent lung metastasis. I propose that the phrase be changed as follows: “The present study demonstrated (...) had the ability to reduce by ~30% the number of lung metastasis of MHCCC97H cells...”

Response: We agree with Dr. Musso’s comment and have revised the phrase.

2. Page 19, Discussion, 2nd paragraph: In “This high efficacy might be explained as less...” The adjective “high” cannot be used here, as a ~30% reduction in the number of metastatic foci is a mild but encouraging result.

Response: We agree with Dr. Musso’s comment and have deleted the adjective word “high”.

Minor Essential Revisions:

1. Figure 6, B-1: in the last two lanes, shouldn’t it read rhFNHC36(100) and rhFNHC36(200)? It seems that “36” is lacking.

Response: This was a careless error. We have corrected it in the new Figure 6.

2. Page 5, Introduction: “Also, the free FN interaction with surface molecules (such as integrins) of liver cancer cells enhanced the viscosity of homogeneous cells”. Please provide a reference for this
statement. It is unclear to the general reader what is called “homogeneous cells”.

Response: This statement is only a speculation about the mechanism of free FN inhibiting liver cancer cell metastasis, which may make readers misunderstood. Therefore, we decided to delete these words.

3. Page 7, Methods: “The yeast expression vector was considered as main factors to locate the clone sites of N-terminal and C-terminal...” I do not understand what the authors mean by this phrase.

Response: We revised this phrase and made it easier to understand the design of PCR primers.

4. Page 10, Methods: What is the size of tumors when they are injected with FN polypeptides or how long after tumor injection polypeptides are injected at the tumor cell inoculation site? This information should be stated in the Methods or in the Results section. Otherwise the reader cannot see whether the approach is preventive or curative.

Response: We have added the relevant information. It is very important for curative application as an approach. In our experiment, a tumor (approximately 5mm in diameter) appeared at the cell inoculation site in each nude mouse model 7 days after tumor cell inoculation.

5. Page 11, MMP activity instead of MMPs activity.

Response: We agree with Dr. Musso’s comment and have revised it.

6. Page 11, “The supernatants were collected … and counted for the remedy of assay”. What does for the remedy of assay mean?

Response: The number of cells in different well (in a culture plate) may be different after 48 hours cultivation, but the volume of supernatant (medium) is the same. To compare the MMP activity that is produced by the same amount of cells in different wells, cell counts should be carried out to determine the final volume of each sample for zymography testing. This procedure is called as remedy assay.

7. Page 13, Statistical Analysis. “… by reliability analysis of SPSS”. The abbreviation should be explained.
Response: We have revised it. ANOVA is the abbreviation of Analysis of Variance and SPSS is the abbreviation of Statistical Product and Service Solutions.

8. Page 14, Results: “… ultrafiltration, ion exchange chromatography and sieve chromatography.”
   Figure 1B should be cited just after this phrase.
   Response: Yes. Figure 1B should be cited just after the phrase “…ultrafiltration, ion exchange chromatography and sieve chromatography”. We have revised it.

9. Page 14, Results: “Western blotting confirmed that rhFNHN29 and rhFNHN36 can be combined with FN polyclonal antibody.” What do the authors mean by “combined”? Do they mean “detected”?
   Response: We agree with Dr. Musso’s comment and have replaced “combined” with “detected”.

10. Page 14, Results: Under the heading Expression Pattern of Integrins and MMPs… the phrase “Different expression of integrins and MMPs demonstrated different abilities…” It is unclear to figure out if the authors are talking about the results in the manuscript (in that case the word “demonstrated” should be changed to “could suggest”) or about previous studies. If the latter is true, the references should be inserted just after the phrase, for clarity.
    Response: We agree with Dr. Musso’s comment and have revised the phrase.

11. Page 15, Results: anti-b3 (BV4) is mentioned twice.
    Response: This is a careless error. We have revised it.

12. Page 20, Discussion, 2nd paragraph: “The mechanism research of tumor invasion and metastasis often correlates the FAK pathway”. The phrase is not clear.
    Response: We agree with Dr. Musso’s comment and have revised the phrase.

13. Page 20, Discussion, 2nd paragraph: “The restoration of p-FAK expression…” This phrase is not clear. If I understood well, I would like to propose: “Expression of p-FAK was found when a tyrosine phosphatase inhibitor (PAO) was added, suggesting that interaction between rhFNHN29 or rhFNHN36 and integrin alpha V beta 3 results in blocking a series of signals into the nucleus
including AP-1 activation.”

Response: We agree with Dr. Musso’s comment and have revised the phrase. Please see the page 21 of the revised manuscript.

14. Page 21, Discussion: “It is suggested that the reasons why rhFN... are: 1) (...). 2) AP-1 transcription factors are leucine zipper proteins...” It is difficult to find a link between point 2 here and the comparison of the effects of FN36 and FN29. Rather, point 2 seems to refer to the differences in the regulation of MMP2 and MMP9 promoters. Please revise or clarify.

Response: Our intention was to explain the phenomenon of Figure 6_B-1 and analyze the reason why rhFNHC36 was better than rhFNHC29 in the inhibition of MMP9 activity. Indeed, we support Dr. Musso’s comment that the statement is not clear, so we revised the phrase.

15. Page 22, Conclusions: “These suggest that rh29 and rh36 may play an important role in safeguarding against human liver cancer and may shed light on a novel strategy for liver cancer therapy.” This phrase lacks precision. I would like to suggest the following phrase: “These findings suggest that rh29 and rh36 may play an important role in controlling human liver cancer invasion and may shed light on a novel strategy for liver cancer therapy.”

Response: We agree with Dr. Musso’s comment and have revised the phrase.

Reviewer, Liliane Gattegno:

I think that this manuscript can yet be published. It has been improved minor comments: The authors may indicate in the material and methods, invasion assay, the different concentrations of peptides or Abs used in the experiments.

Response: We agree with Dr. Gattegno’s comment and have made a supplementary explanation in the methods part. Please see the page 9 of the revised manuscript.

Sincerely,

Yuanzhong Chen and Nanhong Tang
August 5, 2010