Reviewer's report

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

Version: 3 Date: 19 July 2010

Reviewer: orlando Musso

Reviewer's report:

The quality of the manuscript was considerably improved with respect to the 1st and 2nd versions. However, 2 major and 15 minor points still need revision.

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…”
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.

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.
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”.
3. Page 7, Methods: “The yeast expression vector was considered as main facters to locate the clone sites of N-terminal and C-terminal…” I do not understand what the authors mean by this phrase.
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.
5. Page 11, MMP activity instead of MMPs activity.
6. Page 11, “The supernatants were collected … and counted for the remedy of assay”. What does for the remedy of assay mean?
7. Page 13, Statistical Analysis. “… by reliability analysis of SPSS”. The
abbreviation should be explained.

8. Page 14, Results: “... ultrafiltration, ion exchange chromatography and sieve chromatography.” Figure 1B should be cited just after this phrase.

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”?

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.

11. Page 15, Results: anti-b3 (BV4) is mentioned twice.

12. Page 20, Discussion, 2nd paragraph: “The mechanism research of tumor invasion and metastasis often correlates the FAK pathway”. The phrase is not clear.

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.”

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.

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.”

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'