Reviewer's report

**Title:** The role of MMP-1 in breast cancer growth and metastasis to the brain in a xenograft model

**Version:** 1  **Date:** 17 September 2012

**Reviewer:** Joana Paredes

**Reviewer's report:**

Dear Authors,

The objective of this article was to demonstrate that the targeted knockdown of MMP-1 in breast cancer cell models with enhanced brain metastatic ability (231-BR and 231-BR3 cells) was able to reduce primary tumor growth, but also brain metastasis.

The paper is well written and adequate, although there are some important points that need the author's attention and to be clarified, which are detailed below:

**Major Compulsory Revisions**

1) The aim of this paper is based on the results obtained by microarray analyses that were performed by Steeg’s laboratory to identify common differentially expressed genes between the parental MDA-MB-231 breast cancer cells and the variants with brain metastatic ability. The authors argue that altered expression was found for 26 genes in both brain metastasis-derived variants compared with the parental cell line and, of these, MMP1 was the most highly expressed gene. Although this result in not new, it is important to include this data in the present manuscript, or the microarray database where the complete array data can be viewed and accessed. In the present version of the manuscript, there is no indication of a reference where these results can be found.

2) The authors included a variant selected from experimental lung metastases (231-LC3) for comparison, since it did not express increased MMP1. However, MMP1 has been previously shown, by Massagué’s group, to be one of the most important genes linked to the formation of experimental lung metastasis. Indeed, the results of this manuscript also show that MMP1 knockdown is able to decrease lung metastasis. Thus, an explanation for this should be given or discussed in the revised version of the manuscript.

3) In figure 1D, please include the results concerning MMP1 secretion to the conditioned media for sh2 transfected cells. Additionally, the authors should also show the results concerning the 231-BR model for MMP2, TIMP1, TIMP2 and VEGF. Please include the results concerning MMP7, even if negative.

4) Please include some explanation for the discrepant results obtained in migration and invasion abilities of 231-BR and 231-BR3 cells with MMP1 knockdown. Additionally, please refer why there were no significant results concerning sh2 and sh1b for invasion assays, when the MMP1 knockdown shown in Figure 1 was so similar for both shRNA used in each breast cancer
5) Please refer why the inoculation of sh2 cells in mice mammary fat pad was not performed or, if it was, please include the results in the manuscript. It is extremely important to show the results got with sh2, since all the results shown in the paper were performed using only one shRNA sequence (sh1, sh1a, and sh1b).

6) Both models should be used to perform the in vivo experiments that evaluate brain and lung metastasis capacity.

Minor Essential Revisions

1) There are several grammatical errors. Please review the text in order to correct them.

2) Please decrease the details concerning the methodology in the Results section. This description should be restricted to the Material and Methods section.

3) Please improve figure legends.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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'