Reviewer’s report

Title: MMP-9 expression varies according to molecular subtypes of breast cancer

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Reviewer: Jennifer Vandooren

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The authors have evaluated the levels of MMP-9 expression (mRNA and protein) in breast cancer tissues and cell lines. A correlation was found between MMP-9 levels, histological grades of the cancers and a higher incidence of metastasis and relapse.

Major Compulsory Revisions:

A correlation between MMP-9 (gene and protein) expression and breast cancer progression has been found in many studies (including recent ones). The authors should cite these papers and compare their study. For example:

- Van ’t Veer et al., 2002, Nature (PMID: 11823860). (MMP-9 is significantly unregulated in the poor prognosis signature in breast cancers)

- Mehner et al., 2014, Oncotarget (PMID: 24811362). (Production of MMP-9 by tumor cells and progression and metastasis of basal-like triple negative breast cancer)

- Wu et al., 2014, Plos One (PMID: 24845596). (MMP-9 is a potential marker for breast cancer progression)

Background: MMP-9 cleaves denatured collagens (gelatins). This implies that MMP-9 only digests collagens which have first been cleaved by collagenases (e.g. MMP-1, MMP-8,…). See: Van den Steen et al., 2004 (PMID: 15311942) and Rosenblum et al., 2010 (PMID: 20585385). Please adjust this in the manuscript.

The MMP-9 protein expression data solely rely on the detection of immunoreactivity with antibody ab38898, a polyclonal antibody against MMP-9. The authors should test whether or not this antibody cross-reacts with closely related MMPs (e.g. MMP-2). Otherwise, it cannot be excluded that the effect is due to the upregulated expression of a combination of MMPs. In addition, the authors should consider confirming the MMP-9 protein expression data by gelatin zymography (see PMID 23443633).

Figure 1 needs more explanation. For example: define low, intermediate and high MMP-9 expression. What can be concluded from the top representation (colored bars).
Figure 2: The authors state that the MCF7 cell line is the only luminal cell line with elevated MMP-9 levels. However, from figure 2 it can be seen that KPL1 also exceeds the mean expression value. Please address this concern.

Figure 5A: Please add ‘n = ‘ for each molecular subtype and define the p-value for ***.

Figure 6: Please define the p-value for ***

Figure 7: Please label the y-axis

Minor Essential Revisions:

Abstract, Results: remove the red semicolon

Materials and methods: Tissue Microarray, last sentence: (IHC) should be defined immediately after immunohistochemistry.

Results, Overexpression of MMP-9 is associated with a higher incidence of metastases, last paragraph: p-value = 0.001

Results, first line page 16: please correct: ‘As for as the expression...’

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests