Reviewer’s report

Title: The thrombin cleavage domain of osteopontin mediates breast cancer cell adhesion, proteolytic activity, tumorigenicity, and metastasis

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Reviewer: Hwyda Arafat

Reviewer’s report:

In the study by Beausoleil et al, the authors used both in vitro and in vivo studies to show that deletion of the thrombin cleavage site of osteopontin (OPN) can produce changes in a breast cancer cell line that render them less adhesive and more metastatic.

In general the manuscript is well written, however, there are some concerns about the experimental design and data interpretation.

Major compulsory revisions:

- In the adhesion studies (Fig 2) the authors need to also use antibodies against CD44.

- In Figure 1A that shows secreted OPN in the three cell lines, it is clear that there is an extra band above 50 kDa that is secreted by the deleted thrombin cleavage site cells. The authors did not comment on this band and whether it could be responsible for the changed behavior in these cells.

- The authors also did not provide clear explanation or at least proposal for the reason why deletion of thrombin cleavage site of OPN would increase tumor size and metastasis. Is this due to the mere deletion or is it due to actual change in the cell proliferation and migration machinery and for that, in vitro studies and cell proliferation analysis are mandatory to elaborate on this point.

- Finally, the authors conclude that translating their results into the clinic could provide new therapeutic opportunities. My question is this: is deletion of thrombin cleavage site of OPN a frequently encountered phenomenon in breast cancer patients? And if so, how often and does this correlate with more invasive behavior and less survival?

Minor essential revisions:

- It is not clear whether thrombin cleavage site deletion could have secondary effects on the different molecules that can affect the cells’ adhesion and metastatic behavior. For example, what is the status of secreted cathepsin D (CD) levels and activity? In the recent studies by the Sørensen group in JBC 2010, they show that CD cleavage of OPN supported minimal adhesion. It may be important to explore this possibility.
Level of interest: An article of importance in its field

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