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: Paul Shore

Reviewer's report:

The manuscript by Beausoleil et al describes experiments to determine the role of the thrombin cleavage site in Osteopontin (OPN) in breast cancer cells. Over-expression of OPN is associated with metastatic cancers and primary tumors. Understanding the molecular mechanisms by which OPN contributes to tumour development and metastasis is therefore of great importance. Previous studies, including those of the authors, have demonstrated that cleavage of OPN by thrombin leads to an increase in adhesion and migratory properties of cancer cells in vitro. In this study the authors extend their work to examine the role of thrombin cleavage of OPN in vivo. Breast cancer cell lines were generated expressing a mutated form of OPN in which the thrombin cleavage site had been deleted. The functional effect of deleting the thrombin cleavage site on adhesion, uPA expression, tumour growth, metastasis and gene expression was subsequently determined. The authors conclude that “thrombin-uncleavable” OPN confers an early tumor formation advantage and a metastatic advantage on breast cancer cells.

The manuscript is well written, clear and concise. The data are well presented and the experiments have been well performed. The findings are interesting and potentially reveal that inhibiting OPN cleavage leads to an increase in breast tumour growth and metastasis.

Major compulsory revisions

1. As it stands it appears that the authors are suggesting that the lack of cleavage is the reason for the effects. It is clear that deleting the thrombin cleavage domain leads to an increase in tumour growth and metastasis. However, it is not clear whether this is because the deleted version is refractory to cleavage or whether the deletion has had some other effect such as altering the conformation of OPN or inhibiting an undefined interaction. As it stands I am not convinced that it’s the lack of cleavage that is responsible for the observed functional effects. The conclusions could be strengthened by generating point mutations to abrogate cleavage rather than deleting several amino acids.

2. Related to point 1. In Fig. 1 there is an additional band of approximately 55kDa in the deltaTC lane of the OPN western. What is this band? Is it potentially related to the observed functional effects?
Minor essential revision

3. The title needs adjusting. The data do not show that the thrombin cleavage domain of osteopontin mediates breast cancer cell adhesion, proteolytic activity, tumorigenicity, and metastasis. They show the opposite, since deletion of this domain leads to an increase in these properties.

**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