Reviewer's report

Title: Hornerin, an S100 family protein, is functional in breast cells and aberrantly expressed in breast cancer

Version: 2 Date: 2 April 2012

Reviewer: Sandra Z Haslam

Reviewer's report:

General Comments
This manuscript describes the expression patterns of hornerin, an S100 family protein, in normal human and mouse mammary gland, human breast cancers and breast cancer cell lines. Hornerin protein expression levels and cleavage patterns are described that lead the authors to infer an association between increased hornerin expression and apoptosis and less aggressive breast cancers. However, the authors were not able to establish a causal relationship or hornerin-based mechanism based on a stated lack of appropriate reagents.

Major revisions:

Figure 2.
1. It is stated that based on quantitation of hornerin Ab staining hornerin expression was significantly increased during involution. However, it is noted that nuclear hemotoxyin staining is also increased during involution (Fig 2A). In order to accurately quantify homerin staining without introducing the nuclear stain artifact it is necessary to do quantitation on sections that are not counterstained. Otherwise it is necessary to convince this reviewer that nuclear stain is not contributing to the interpretation of increased hornerin expression.

2. In figure 2C the dark staining cells are referred to as macrophages. How were these cells identified to be macrophages – staining with a macrophage specific marker is needed.

Minor comment: Introducing LPS stimulated blood derived monocytes does not appear to be relevant.

Figure 3.
1. For 3C Western blot quantitation: the bands for 245KDa intact and 100KDa fragment of hornerin need to be normalized to tubulin expression and ratio of hornerin:tubulin should be plotted and not “relative units". The only correct conclusion based on statistical analysis is that the 245KDa was differentially expressed and not the 100KDa fragment. The wording in results needs to be changed.

Figure 5
Minor - What expression (RNA, protein) was measured to establish no difference
for receptor status among the various cell lines? Please clarify in the text.

1. Fig 5C. It is not clear what the 2 panels each for N-term and C-term are and what they are intended to show. Please explain. For the MDA MB231 and SUM 149 images it appears there is significant cytoplasmic staining - how are membrane and cytoplasmic staining distinguished? Need to describe.

Discussion

Minor comment: Too much of the discussion refers to other findings that are speculatively used to support the putative association of hornerin expression with apoptosis/necrosis and less aggressive breast cancers.

**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.