Reviewer's report

Title: Large-scale proteomic identification of S100 proteins in breast cancer tissues

Version: 3 Date: 26 April 2010

Reviewer: Kjetil Boye

Reviewer's report:

The present study by Cancemi et al describes a proteomic approach to identify the presence and relative expression levels of several S100 proteins in a panel of tumor samples from one hundred breast cancer patients, as well as the association of S100 protein expression with clinical parameters and patient outcome. The data are interesting, both as a contribution in the search for novel biomarkers to assist in clinical decision-making, and to better understand the role of S100 proteins in breast cancer biology. There are, however, a number of issues that need clarification.

Major Revisions

1. I am concerned with the quantification algorithm used. If I have understood the algorithm correctly (page 7), the spot volume (integrated dose intensity over the spot area) for one specific spot (e.g. S100A7) is divided by the sum of integrated dose intensities for all spots on the gel to obtain the value designated "%Vol". Thereafter, this value is divided by the %Vol value for actin to generate the value N%V. If this is correct, the spot volume is first normalized against the sum of spot volumes on the gel to correct for differences in gel staining, but then divided by a value (Actin %Vol) that is also normalized against the sum of spot volumes on the gel. Hence, the value N%V is equal to the spot volume of one specific spot divided by the spot volume for actin, and thus does not correct for differences in gel staining.

2. This study is focused on the distribution of S100 protein expression in 100 breast cancer patients and association with patient outcome. Therefore, baseline patient characteristics (conventional clinical and histopathological parameters) must be presented, including the time period of inclusion for this study. Additionally, follow-up of patients must be described (e.g. how was metastatic disease defined, which radiological investigations were performed to detect metastatic disease, were the patients followed by the surgeons or their general practitioner, etc).

3. Validation of at least one of the differentially expressed S100 proteins in a set of patient samples should be performed, and this could easily be accomplished by immunoblotting using specific, commercially available antibodies (such as for actin in Fig 1).

Minor Revisions
1. It is not clear whether the present study also include patient samples/results from the previous studies from the same group (such as ref 24). This should be clarified.

2. In the section "Clinical specimens" (page 5) the authors refer to bioethical recommendations. The name of the bioethical committee and the approval number should be stated.

3. In page 7, last line, the authors state that an area covering a pl/kDa range of 4-8/15-9kDa was analyzed, and the figure text states 4-6.5/15-9kDa, but the figure shows a pl of 4.5-7 and a Mw of 14-9 kDa. What is correct?

4. In Fig 3, no mass spectrum of spot 8 is shown, and two spectra are shown for spot 10.

5. Fig. 5 is difficult to read and should be presented in a different way, such as a table with number of patients and percentages in the different categories.

6. Statistical methods that incorporate the time period from surgery to development of metastatic disease are more suitable for analysis of patient outcome, such as Kaplan-Meier plots and log-rank test, and such analyses should be performed. The data and statistical method presented in Fig 8 are valid, but much information is lost when the time factor is not included.

7. Correction for multiple testing should be performed in Fig 7.

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

**Quality of written English:** Needs some language corrections before being published

**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