Reviewer’s report

**Title:** Qualitative and Quantitative Proteomic Analysis of Nipple Aspirate Fluid from Women with Early-Stage Breast Cancer using Isotope-coded Affinity Tags and Tandem Mass Spectrometry

**Version:** 1  **Date:** 29 November 2005

**Reviewer:** Hon-Chui Eastwood Leung

**Reviewer’s report:**

**General**

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**Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**

1) The authors need to perform a reciprocal labeling to validate the list of differential proteins. This could confirm if the same list of proteins showed up even in reciprocal labeling.

2) No statistical analysis on the ratio of heavy:light peaks was shown, therefore, one might question if a difference with less than two-fold in expression was statistically significant.

3) The authors should show results from a validation immunoassay that correlated with ICAT finding in couple markers.

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**Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

1) The minority of the cancer group were post-menopausal patients. However, the control group of normal pre-menopausal volunteers was missing. The ratio of pre- and post-menopausal patients should be similar.

2) Number of singleton peaks was not listed. They might be the better markers to use with bigger difference in expression than the marginal two-fold in the listed markers.

3) Proteins at the bottom of table 1 were not significant. ProICAT score under 29 could be regarded as non-specific identification even in 99% confidence level. Protein identified using one peptide is regarded as false positive generally, see reference by Adamski et al 2005 in Proteomics 5:3246-61. Even with manual curation, one may need to use Mascot as a second filter after ProICAT to eliminate the false positive identification. Heavy:light ratio based on two pairs of peptides could also cross validate the finding of heavy:light ratio in one pair of peptides.

4) The authors need to give an account on the omission of other known cysteine-containing breast cancer proteins from nipple aspirate such as i) PSA and ii) gross cystic disease fluid protein 15.

5) Was it optimized by scanning only the two most intense ions in MS/MS analysis?
6) Page 11, the statement that “menopausal status did not affect the expression of vit-D binding protein…” was not valid. If the P value was really 0.57, then the conclusion was not substantiated by the statistical analyses. Even though it was a typo, the authors should screen all the samples before conclusion instead of the limited number in the Western blot shown in Figure 5.

7) The legend of Figure 5 was missing.

8) There were three proteins in Table 1 in which the ratio was 999. Did it mean a singleton peak or were they typos?

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.

Eastwood Leung
29th Nov. 2005