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: 25 November 2005

Reviewer: Antonia Vlahou

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)

In this manuscript the authors address the identification of biomarkers for breast cancer by the application of the ICAT methodology in the protein analysis of nipple aspirate fluid (NAF). Cancer and normal paired samples from women with unilateral breast cancer were analyzed. Several proteins were found to be differentially expressed; among those the differential expression of Vitamin-D binding protein was further investigated and confirmed by Western blotting. Overall, this is an interesting study demonstrating the application of quantitative proteomic approaches in the investigation of the NAF proteome. Nevertheless, to increase the validity of the results presented herein, the following points will have to be addressed, prior to the publication of the manuscript.

1) In the list of proteins presented in Table 1 several were identified based on 1 peptide. These identifications may be regarded unacceptable, particularly considering that the criteria utilized during the database search are not described in the manuscript. The criteria and specifically, number of miscleavage/s allowed, ppm accuracy or MS and MS/MS tolerance, and allowed modifications should be provided. In addition the method of calibration should be described. Furthermore, it is recommended that the molecular weight as well as the % coverage of the identified proteins are provided in Table 1.

2) Some of the ratios described in Table 1 (for example 1.01, 1.05, 0.92) appear to be not indicative of differential expression. Usually in these methodologies, a cut-off of 15-20% change is utilized for differential expression. The authors should elaborate on the variability of the methodology and provide the criteria they used to assemble the list of differential expressed proteins.

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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)

In the background the sentence "This is mainly because MS is not an inherently quantitative technique and does not allow for the specific identification of individual peptides" should be rewritten. The first part of the sentence is true (i.e. MS is not quantitative) but the second part is not-MS allows for protein/peptide identification.

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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.