Reviewer's report

Title: Differential Proteomic Comparison of Breast Cancer Secretome Using a Quantitative Paired Analysis Workflow

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Reviewer: Sharon Pitteri

Reviewer's report:

This manuscript describes a proteomic study of paired nipple aspirate fluid samples from patients with unilateral breast cancer. The study is focused on the breast cancer secretome that contains proteins enriched in the tumor microenvironment. A novel bioinformatics software, PatternLab, is used to assess peptide level differences with disease state. 88 proteins are identified as being differentially abundant and the biological origins of these proteins is described. Overall the paper is interesting and the strategy to use nipple aspirate fluid and perform pairwise studies to identify breast cancer biomarkers is attractive and highly complementary to other biomarker studies in the literature. I would recommend that the following points be addressed:

1. The paper focus of the paper is slightly convoluted because a new bioinformatics tool is described and the tool is applied to the nipple aspirate fluid analysis. Is this the first time that PatternLab has been described in the literature? If so, it would be good to further qualify the tool. For example, the information obtained by traditional, more standard/established, proteomics analysis tools such as Proteome Discoverer should be compared to the information obtained from Pattern Lab.

2. There are many points in the sample collection that need further clarification. Although the NAF collection has been previously described, a brief description of how the samples were collected should be given here. It says that the eligibility criteria included that all subjects were "to be on menopause." Does that mean the subjects were currently menopausal? Given the ages, this is probably not the case. Perhaps this means that the subjects were post-menopausal? In Table 1, "Birth control use" needs to be clarified. Does this mean they every used birth control? What kind of birth control? The same comments apply for hormone replacement. Also in Table 1, how is breastfeeding defined? Is there a minimum duration of breast feeding? Lastly in Table 1, why were samples collected from the three individuals without breast cancer? Did they have some other benign lesion?

3. In the sample preparation, how was protein extracted from the nipple aspirate fluid?
4. The differentially abundant proteins between cancer and non-disease breasts included 87/88 proteins that were not in the Plasma Proteome Database. These proteins look like a proteomic profile of plasma. Are the nipple aspirate samples bloody? If so, this would be a huge confounding factor in the analysis. Also, the results that that several immunoglobulins are not included in the Plasma Proteome Database. That seems very strange given that immunoglobulins are a major component of plasma proteins.

5. The validation, of even a single protein, by an orthogonal method such as Western Blot in cancer/normal nipple aspirate fluids would really boost the confidence in the findings in this paper.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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I am able to assess the statistics

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