Author's response to reviews

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

Authors:

Timothy M Pawlik (tpawlik1@jhmi.edu)
David H Hawke (dhawke@mdanderson.org)
Yanna Liu (yliu@mdanderson.org)
Savithri Krishnamurthy (skrishna@mdanderson.org)
Herbert Fritsche (hfritsche@mdanderson.org)
Kelly K Hunt (khunt@mdanderson.org)
Henry M Kuerer (hkuerer@mdanderson.org)

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Author's response to reviews: see over
RE: REVISED MANUSCRIPT MS: 6144244858247860
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

Dear Dr. Newmark:

We would like to thank you and the reviewers for your careful review of our manuscript. We are pleased that, in general, the manuscript has been very favorably reviewed and that it has been recommended for publication by two reviewers with either no or only minor revisions. We have specifically addressed each of the reviewers’ comments below and have revised the manuscript accordingly.

Reviewer 1:
General Comments
No revisions requested. Recommend accept without revision.

Reviewer 2:
General Comments
No major revisions. Recommend accept after minor revisions.

Specific Comments
1. The description of the criteria utilized during the MS analysis should be reported in the text, not only in the response to the reviewers.
   The description of the criteria utilized during the MS analysis has now been included in the text of the Methods section on page 9 of the revised manuscript.

2. The authors still have not corrected the title of Table 1 which generates confusion for the reader.
   We have changed the title of Table 1 to read “List of proteins identified with reasonable certainty in order of relative strength of assignment.”
Reviewer 3:

General Comments
A number of revisions need to be addressed prior to publication.

Specific Comments
1. The authors did not show validation assays of other markers, the title should therefore only be limited to “up-regulation of vitamin D binding protein in breast cancer patients.” The use of the word “proteomic analysis” is not appropriate as the standard for proteomic analysis includes validation of differential markers.
   We respectfully disagree with the reviewer. Proteomic analysis refers to the technologies employed, not necessarily the results. In the current study, we did indeed find a “differential marker” and provided evidence of its validation. We are not adamantly against a change in the title of the manuscript if this would otherwise preclude publication, but do not think a title change is warranted.

2. A more detailed analysis of the Western blot results should be presented.
   Further analysis of the Western blot results were not requested by either of the two other reviewers. Similarly, we do not believe that additional analyses are warranted. The presentation of the Western blot results are consistent with what is usually reported.

3. It seems as if the authors did not address the questions about missing cysteine containing peptides and finding one peptide of Vit D binding protein without cysteine residue point adequately. The questions about only checking on the two most prominent peptides in MS/MS also suggest that the authors may miss a list of potential differential biomarkers.
   We respectfully disagree with the reviewer. First, we would not expect to observe even most of the Cys peptides unless there was a very large quantity of the protein present in the analysis. Not likely when searching for a biomarker. Second, finding a peptide from this protein which does not contain a cysteine does not relate at all to the derivatization, since that peptide was not derivatized and did not contain a cysteine. The point of mentioning it was to support the assignment of the protein; otherwise it would be a one-peptide hit. The reason we found it despite the fact that it lacks a Cys and was not labeled, is that the affinity separation step (thankfully, in this case) is not 100.000% selective, and could not be expected to be. Third, the suggestion that this report only addresses the “two most prominent (sic) peptide (sic) in MS/MS” is unreasonable and suggests a misunderstanding of the experiment on the part of the reviewer.

4. Why are we still getting Figure 3 and 4 but not Figure 3a and 3b?
   As noted in our previous letter, there appears to be a problem with the electronic submission process that does not allow for the submission of sub-set figures (i.e. Fig 3a and Fig 3b). We will work with BMC and the editor to correct this problem with the submission.

5. The ratio “999” still appears in the footnote of Table 1 and should be labeled “N/A”.
   We apologize. The reviewer is correct. We have changed the footnote to read “N/A.”
Thank you in advance for your time in considering our revised manuscript.

Sincerely,

Timothy M. Pawlik, MD, MPH
Assistant Professor
Department of Surgery
Johns Hopkins Hospital

Henry M. Kuerer, MD, PhD
Director, Breast Surgical Oncology Training Program
Associate Professor of Surgical Oncology
The University of Texas M. D. Anderson Cancer Center