Author’s response to reviews

Title: CXCR4 expression on circulating pan-cytokeratin positive cells is associated with survival in patients with advanced non-small cell lung cancer

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BioMed Central Cancer

Re: Revised Manuscript—CXCR4 expression on circulating pan-cytokeratin positive cells is associated with survival in patients with advanced non-small cell lung cancer

Dear Editor:

We would like to thank the reviewers for their insightful comments on our manuscript, “CXCR4 expression on circulating pan-cytokeratin positive cells is associated with survival in patients with advanced non-small cell lung cancer.” The manuscript has been thoroughly revised in accord with the reviewers’ suggestions. Please find below point-by-point responses to each of the reviewers concerns. The modifications are formatted to display the reviewer’s comment first followed by the revision. The revised manuscript has been reviewed and approved by all of the authors, and as such, submitted for your timely review.

Reviewer 1:

1. The experiments and the data may be right. However, the interpretation for the data seems insufficient.

   Thank you for this observation, as stated below in #3, we have updated the statistics.

2. Abstract, Methods—“Pan-cytokeratin positive cells were increased in the circulation of patients with NSCLC, as compared to normal control subjects.” This is not method.

   This statement was moved to the Results section of the abstract.

3. Statistical Analysis—“Cox proportional hazards models investigated the effect of high or low CXCR4 levels on survival. Hazard ratios, 95% confidence intervals, and p values were determined for each group and a Kaplan-Meier curve was produced.” There was no Cox model analysis in this manuscript. The authors need to enlist the services of a talented biostatistician to perform the appropriate analyses and determine which of the multiple factors independently influence prognosis.
We have updated the statistical analysis to include a log-rank test for the survival comparisons. Group comparisons were made with a Student’s t-test. A Cox model was not used, and due to the small number of patients in this study a multivariate analysis using prognostic factors was not performed.

4. Results and Figures—Page 7 and Page18, “p < 0.05”; Figure 2 “p = 0.05” Which is correct?

The p-values correspond to the comparison of pan-cytokeratin expression between cancer and normal controls and and combined pan-cytokeratin/CXCR4 expression between cancer and normal controls, respectively as stated in the text. The single and double astericks in Figure 2 denote the different comparisons and corresponding p-value.

5. Figure 2—How many cases did author examine in the experiment of Figure 2? Twenty-eight patients and 14 donors, or 13 patients and 6 donors? Are the bars standard deviations or standard errors?

The patient information for Figure 2 has been clarified on page 7. The text now states, “To determine CXCR4 expression on mononuclear cells expressing pan-cytokeratin, we measured human cytokeratin 14, 15, 16, and 19 (pan-cytokeratin) along with CXCR4 on peripheral blood mononuclear cells (PBMC) by FACS in 28 NSCLC patients and 14 normal controls.”

Page 18, Figure legend for Figure 2 has been changed to, “Circulating pan-cytokeratin positive cells and CXCR4/pan-cytokeratin positive cells in 28 patients with NSCLC and 14 normal subjects.”

The error bars represent standard deviations.

6. Figure 3—The scale of the vertical axis should be changed, for example, upper limit should be 15000, so that the difference of positive cells among the groups should make easy-to-understand.

Figure 3 has been updated.

7. Figure 4—Log-rank test is necessary to compare survival curves.

Figure 4 has been updated and a log-rank test has been used to compare survival between groups.

8. Table 1—It should be more sophisticated.

Table 1 has been updated.

9. Discussion—Fourth paragraph, Fifth paragraph, and Conclusions have no correlation with author’s experiments.
The fourth and fifth paragraphs have been updated to discuss other biomarkers for evaluating micrometastatic disease in lung cancer. The conclusions have been edited.

Reviewer 2:

1. The introductory material seems lacking of the information of cytokeratin and the significance and value of this traditional marker in diagnosis of non small-cell lung cancer.

   A description of the use of cytokeratin as a marker for circulating tumor cells has been added to the Background section, second paragraph on page 3.

2. According to Pan’s report (Mol Cancer 2006, 5:56), the expression of CXCR4 on circulating pan-cytokeratin+ cells in mRCC (renal cancer) patients was more than 1#106 cells/ml, why this expression in NSCLC patients in this study was low around 2500 cells/ml (Fig 3)? If CA07 patient held very high levels of double positive circulating cells compared to other patients, is this level correlated with much more progressed disease stage or less survival?

   The expression of pan-cytokeratin/CXCR4 was lower in our patients with NSCLC than in the previous study in RCC. Since only one patient demonstrated very high levels, we could not perform any correlates or statistical analysis on survival based on one case.

3. Instead of discussing signaling pathways involved in the regulation of CXCR4 expression and the metastatic potential of tumors which was not studied in the current work, the discussion should refer to several other reported biomarkers for metastatic lung/other cancer cells such as adhesion molecules (Ep-CAM, CD44), metalloproteinases inducer (EMMPRIN), chemokine receptors (CCR6, CXCR4) and epithelial differentiation marker including p63, MOC-31, thyroid transcription factor-1 as well as their distinct diagnostic utility.

   The fourth and fifth paragraphs have been updated to discuss other biomarkers for evaluating micrometastatic disease in lung cancer.

Thank you for your careful consideration of this revised manuscript for publication in BioMed Central Cancer.

Sincerely,

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