Author's response to reviews

Title: Transformation of human bronchial epithelial cells alters responsiveness to inflammatory cytokines

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Author's response to reviews:

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BMC-Cancer

Re: MS: 6110777437520870 - Transformation of human bronchial epithelial cells alters responsiveness to inflammatory cytokines

Dear Editor,

My colleagues and I were pleased to learn the very positive review by Dr. Frank Buhling and would like to thank him for the helpful suggestions for revision. We have modified the manuscript by integrating the recommendations given in the reviewer's report. The revisions respond to the points raised as follows (first, with quote marks, the reviewer's individual comments, then, our response with the listing of the specific changes made in the manuscript):

"Minor Essential Revisions"
"Table 2: It is not clear how many cultures were obtained and used: There are 110 successful brushings (in line 3) but overall 113 cultures (from lines 6-14)?"

We have missed to enter the new number for the total of cultures at the last update. The number has been corrected along with the numbers given in the Result section on page 11, third paragraph.

"Figure 1 A) Control blots which document equal protein load which are stained with antibodies to total Erk or STAT proteins should be displayed in Fig. 1a."

As part of the analyses of the cell lines, the suggested immunoblots of total STAT3 and ERK had been carried out. The pictures were not included in the first version of Fig. 1 in an attempt to keep the presentation simpler. We fully agree with the reviewer about the value of an internal marker for loading as way to improve the scientific presentation. We have added now the images for total ERK in Figure 1A. An appropriate technical note has been added to the Method section on page 8, end of the third paragraph.

"B) The cell lines which have been investigated in Fig. 1 represent a relatively homogenous model and the authors have performed more than 20 independent preparations. Therefore, it would be interesting to see the summarized results of these reactions (after densitometric evaluation as described in Materials and methods). From this it should be clear whether the changes which are shown in the blots are statistical significant."

The statement about "20 independent preparations" referred only to the type II epithelial cells and had been made to indicate that we have characterized a sufficient number of cell cultures to know the cytokine response profile that defines this cell type. A side-by-side characterization of epithelial cells and fibroblasts, which were derived from the same patient's lung specimen and had been analyzed as shown in Fig. 1A, were limited to 5 cases. We have clarified this in the legend to Fig. 1 to indicate that the presentation of dat
is limited to 5 cell preparations. The quantification of the patterns have been done as described in the Method section on page 8, third paragraph, and, as suggested by the reviewer, the values (mean +/- SD, N=5) are now presented in the Fig. 1, new panel B. The findings are mentioned in the Result section on page 9, third paragraph. The reviewer is correct in that the use of the cell lines will produce data with much lower variability than primary cultures. Because the magnitude of the signaling caused by the cytokine treatments is so large, the significance of the differences should now be evident from the data (mean + SD) as given in Fig. 1B.

"Discretionary Revisions"
"Figure 4 This figure is important but very complicated! I would suggest to re-evaluate the outline. The differences between paired samples could be displayed more impressive if they would be compared directly. For example the ratios between "normal" and "abnormal" samples could be displayed for each parameter. Using this approach it would be more clear whether they are increased or decreased."

Figure 4 is the heart of the paper and presents the major findings of the study. Two key messages of this figure are: (1) the description of the response pattern of primary epithelial cells and (2) the variability of this pattern among individuals. In the Introduction (page 4, third paragraph) and in the Result section on page 11, second paragraph) we have stated our goal to identify right these points. The presentation of ratios, as suggested by the reviewer, will indeed emphasize the transformation-associated changes, but it will eliminate the description of the response patterns. As mentioned by the reviewer, we also had noted that the calculation of the ratios would highlight the effects of transformation. In response to the reviewer's suggestion, we have added two new panels, G and H, to Figure 4, which present for each paired cultures the ratios (increases and decreases) of the most relevant treatments that affect ERK and STAT3. Appropriate reference to these new panels is included in the Result section, page 13, first paragraph, page 14, second paragraph and Discussion on pages 17 and 18.

We hope by these revisions the manuscript will meet your and the reviewer's approval.

In the name of all my colleagues, I wish to express my thanks to you and the reviewer, Dr. Frank Buhling for your efforts.

Sincerely yours

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