Author's response to reviews

Title: Macrophage traits in cancer cells are induced by macrophage-cancer cell fusion and cannot be explained by cellular interaction

Authors:

Ivan Shabo (ivan.shabo@liu.se)
Kristine Midtbø (krimi000@student.liu.se)
Henrik Andersson (Henrik.A.Andersson@lio.se)
Emma Åkerlund (emma.akerlund92@gmail.com)
Hans Olsson (hans.olsson@lio.se)
Pia Wegman (pia.wegman@lio.se)
Cecilia Gunnarsson (cecilia.gunnarsson@lio.se)
Annelie Lindström (annelie.lindstrom@liu.se)

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Author's response to reviews: see over
To the Editor of BMC Cancer

Dear Sir,

Thanks for your and reviewers comments of our article entitled “Macrophage traits in cancer cells are induced by macrophage-cancer cell fusion and cannot be explained by cellular interaction”. The manuscript is revised according to your request as follows:

1- All changes in manuscript text is done in red text.
2- Figure 2 and 5 are revised according to the following discussion.
3- Below is our comments to the reviewers

Reviewer: Lill-Tove Busund
Major Compulsory Revision
- CD163 staining of CD163 expression in breast cancer cells was assessed by an experienced pathologist (Hans Olsson MD PhD) and a experienced breast surgeon (Ivan Shabo MD PhD) with interest in breast cancer histopathology. During the IHC analysis, we were aware that it is important to distinguish between cancer cells and macrophages. Using double-IHC staining was an option that we discussed during the study. Because we used strict cytomorphic criteria and considered this as reliable to distinguish between macrophages and cancer cells, we chose not to use double-IHC.

The following sentence was added in the materials and method: "Macrophages and cancer cells could be distinguished on morphological basis. Macrophage nuclei were small and regular, whereas the cancer cells were atypical with pleomorphic nuclei and enlarged".

- Magnification of histological pictures is now added in legend for figure 5. (magnification of ×200)
- Explanations about the images in Figure 5a are corrected. The purpose of the red arrow in upper picture is to show how CD163 positive cells grow in groups. In the bottom image, the red arrow show CD163 positive cancer cells with large pleomorphic nuclei compared with CD163-negative cells (blue arrow). Note that the nearby macrophages morphologically clearly differ by less cytoplasm and smaller nuclei.

Minor essential Revisions
- Abbreviation GFP is explained in manuscript text.
- The concentration, type and isotype controls are stated in M&M section.
- The aim of presenting the results of STR analysis was to demonstrate the similarity of STR DNA profile, such as the presence of the Y chromosome, between MCF-cells, macrophages and their corresponding hybrids. Therefore, we
propose to display the results of STR analysis in a table. I enclose below a Venn diagram (corresponding to the results in Table 1) as an presentation to the editor.

- The hybrids were grown for at least 7 days. They changed phenotype over time and depending of the micro-environment in which they grew. These findings are under ongoing investigation in our laboratory.

**Reviewer: Nathalie Scholler**

**Major Compulsory Revision**
- **Figure 2:**
  Figure 2, we have redesigned. Data on negative and isotype control are shown in Figures a - d. Figure 2 legend is rewritten with the clarification regarding the negative control.

- **Figure 3:**
  In line with almost all immunofluorescence images published in BMC Cancer, we omitted showing bright field and isotype controls. We believe including bright filed in the merged images does not make them more informative, rather the opposite. Negative stainings can be seen for the CD163 antibody on MCF-7 cells and for GFP of course there is no isotype since no antibody was used. Please note that negative controls is clearly shown at FC analysis. We therefore propose to maintain figure 3 unchanged. As a presentation to the editor, we added merged images to the figure and these are shown in the end of this document.
- **Figure 4**
  This issue is explained in previous comments to feedback from Reviewer LT Busund.

**Minor essential revision**
- **Figure 1:**
  There is no exclamation marks in Figure legend.
- Cell sorting is described in detail by M & M, under the heading *Fluorescence-activated cell sorting (FACS)*. Sorting of hybrids is based on the co-expression of macrophage phenotype (CD45 and CD163), and GFP.

**Discretionary revisions**

We have ongoing studies in which the cellular behavior of hybrids in relation to their parent cells is ongoing. Hybrids between the other cancer cell lines and macrophages is described in previous studies, both in vivo and in vitro.

![Figure 1: Images of Macrophages, MCF-7, and Hybrids showing CD163, GFP, and Merge](image)

Sincerely

Ivan Shabo MD PhD
Consultant in Endocrine and Sarkoma Surgery
Dept. of Surgery
Institution of Clinical and Experimental Medicine
Linköping University Hospital
581 85 Linköping
Sweden