Author's response to reviews

Title: Mononuclear cells support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator

Authors:

Philip Vlaicu (vlaicu@biochem.mpg.de)
Philipp Mertins (pmertins@broadinstitute.org)
Mayr Thomas (mayr@biochem.mpg.de)
Peter Widschwendter (peter.widschwendter@med.uni-tuebingen.de)
Beyhan Ataseven (beyhan.ataseven@swmbrk.de)
Bernhard Hoegel (bernhard.hoegel@t-online.de)
Wolfgang Eiermann (wolfgang.eiermann@swmbrk.de)
Pjotr Knyazev (knyazev@biochem.mpg.de)
Axel Ullrich (ullrich@biochem.mpg.de)

Version: 2 Date: 26 March 2013

Author's response to reviews: see over
Author’s response to reviews

Title: Mononuclear cells support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator

Authors: Philip Vlaicu, Philipp Mertins, Thomas Mayr, Peter Widschwendter, Beyhan Ataseven, Bernhard Högel, Wolfgang Eiermann, Pjotr Knyazev, Axel Ullrich

Version: 2 Date: 26 March 2013

Author’s response to reviews: see over
1. The authors should make distinction of M1- and M2-polarized TAMs that have contrasting effects on anti-tumor immunity.
   - Our *in vitro* differentiated macrophages express high levels of the scavenger receptor AI and of CD14 (Figure S1). This expression pattern is characteristic of M2 macrophages [1], associated with the progression various carcinomas *in vivo* [2-4]. The discussion section has been changed.
   - We shed light on one important aspect of the complex interactions between monocytes/macrophages and tumor cells.
   - We clearly show that undifferentiated PBMCs as well as spontaneously differentiated macrophages are primed by tumor cells to secrete EGFR- and STAT3-activation factors. These findings are corroborated and expanded by our study of IHC samples from breast cancer patients.

2. Tumor secreted factors should be identified in each cell line used. It is expected that different cell lines secret different factors and consequently have differential priming capability towards monocytes and macrophages.
   - Investigating the priming factors secreted by the different cell lines used is a very interesting undertaking, but not within the focus of this paper. Indeed, some ligands capable of priming macrophages have been described ([5] and manuscript references 12, 26).
   - We have focused on the finding that monocytes/macrophages answer in a very stereotype manner to priming by tumor cells. Not only do monocytes/macrophages secrete factors that promote tumor progression, but elevated systemic concentrations of one of these factors are of diagnostic value, highlighting aggressively growing tumors (Figure 4).

3. Table. MCF10-A is a benign cell line. The authors should provide explanation why and how the cells can prime PBMC cells to secret EREG and OSM.
   - MCF 10A cells share a number of secreted proteins with breast carcinoma cell lines [6]. Among these proteins is macrophage migration inhibitory factor (MIF), which promotes the alternative activation of TAM towards an immunosuppressive, angiogenesis-supporting state [6, 7].
   - MCF 10A have spontaneously immortalized after being isolated from breast tissue that displayed extensive fibrocystic disease, dilated mammary ducts, benign apocrine metaplasia as well as intraductal hyperplasia. Nevertheless, the cells are not tumorigenic and invasive *in vivo* and are thus classified as a benign cell line [8].

4. In Fig. 2B and 2E western blots, total EGFR and total STAT3 levels should have been determined.
   - EGFR and STAT3 have been used as loading controls in Figures S6A and S6D, respectively, and correlate with tubulin levels. Tubulin was used further
on as loading control, as EGFR and STAT3 levels did not change significantly during the short stimulation times used in our system.

5. In Fig 3 migration assay, the identity of the receptors expressed in the tumor cells needed to be investigated.
   • According to literature, MCF7 and MCF 10A cells express EGFR protein [9, 10]. Figure S6A shows SCC-9 cells to express EGFR protein. Figure S2 shows all three cell lines to express oncostatin-M receptor mRNAs, i.e. gp130 and OSMRβ. The results part has been changed accordingly.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests.
Reviewer's report
Title: Mononuclear cells support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator
Version: 1 Date: 24 January 2013
Reviewer: Lalita Shevde-Samant

Reviewer's report:
This manuscript details the impact of the TMAs on the tumor cells and vice versa. It presents a very detailed assessment of the cytokines/growth factors that are produced by the two cell populations as a result of these interactions and the ultimate impact on tumor cell behavior. The data on HB-EGF is very well supported by clinical data as well.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report

Title: Mononuclear cells support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator

Version: 1 Date: 25 January 2013
Reviewer: Margit Janát-Amsbury

Reviewer's report:
Section of materials and methods is well described. The authors provide detailed info in supplementary part.
All data is linked well to the results and experimental setups contain relevant internal controls. Nothing is exaggerated.
Discussion and conclusions are balanced and well linked with clinical findings to support the data.

1. However, the title is questionable. Definition of mononuclear cells means monocytes and lymphocytes, but this study mainly focuses on cancer associated-monocytes/macrophages omitting findings on lymphocytes. This term is frequently used throughout this paper. The title probably needs a minor change. The usage of the term 'mononuclear cell' should be more specific.
   - We have changed the title accordingly to “Monocytes/macrophages support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator”. We have introduced the term „monocytes/macrophages” in the manuscript to distinguish these cells from lymphocytes.

2. The results section would benefit from being streamlined and link more logically to an order the experiments were conducted/presented
   - We have made changes to the results and discussion sections.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
No competing interests
References


