Author's response to reviews

Title: Gene expression analysis after receptor tyrosine kinase activation reveals new potential melanoma proteins

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Author's response to reviews: see over
Dear Dr Xin Chen and Miss Angelina Ilievska, MSc,

Herewith I submit the revised version of our manuscript with the number 1676662685379605 (Gene expression analysis after receptor tyrosine kinase activation reveals new potential melanoma proteins).

We have addressed the concerns raised by the reviewers and have complied their requests.

Please find below our point-by-point response to the requests and suggestions from the reviewers:

Referee 1: Rodney S Nairn

1. Major compulsory revisions - none.
2. Minor revisions - Although well written, in some spots the English usage could be improved: e.g., 3rd paragraph of Background, 1st sentence "By contrast" instead of "contrarily".
6th paragraph of Results, 4th sentence "... the gene was found to be upregulated..."
9th paragraph of Results, 1st sentence "effects ... were abrogated..."
11th paragraph of Results 3rd sentence "At the level of protein..."
12th paragraph of Results last sentence "... the extent and time course of stimulation were comparable..."
Discussion, 1st paragraph: Why are there hyphens after MAPK and MEK?"

- We thank the reviewer for the linguistic improvements. We have corrected the respective sentences.

"3. Discretionary revisions - I don't like the way the Discussion ends with one sentence. This could be extended by emphasizing that the work shows proof-of-principle for using the system developed for discovery of new gene targets in melanoma; also some concise point concerning the FOSL1 result and its importance would be appropriate

- We have now extended the last part of the discussion and have emphasized the usage of Xmrk as a tool in discovering new melanoma-relevant proteins.

“In summary, we used the high overlap between pathways downstream of Xmrk and established human melanoma pathways for the search of new melanoma-relevant target genes. Our gene and protein expression results indicate that Xmrk serves as a suitable model oncogene for this purpose. As a proof of principle, we investigated the AP-1 complex component FOSL1 in more detail. We found that the gene is similarly regulated in a MAPK-dependent manner by Xmrk and by human melanoma oncogenes. Importantly, we also could demonstrate a pro-tumorigenic role of FOSL1 in human melanoma cell lines, thus confirming the Xmrk oncogene as instrumental in the search of new melanoma players”
Referee 2: Rutao Cui

Major points

“1. All data were collected from in vitro. The conclusion will be much more solid if the author can show the FOSL1 expression in human melanoma tissues.”

- We agree that this is a very important point. With the new version of the manuscript, we are providing a link to the human protein atlas, which supplies expression data for many proteins in a large amount of normal human tissues, tumor tissues and tumor cell lines (http://www.proteinatlas.org/tissue_profile.php?antibody_id=4396&g_no=ENS G00000175592). Melanoma tissue and cell lines are also included, and most of them show moderate to strong FOSL1 expression, while epidermal skin only displays moderate expression. Most normal tissues, however, express low or no levels of the protein, while, in addition to melanoma, other tumor types also show strong FOSL1 expression. This indicates that strong FOSL1 expression occurs predominantly in transformed cells, including melanoma.

We have included this information into the last paragraph of the Results section.

2. As a potential marker, is FOSL1 specific expressed in melanocyte lineage? If not, the significance of this study will be concerned. In melanoma clinical, the dermatopathologists usually use some specific marker (S-100, HMG-45 or Mert-1/Melanin A) to recognize the melanocytes only.

- Expression of FOSL1 is not exclusive to the melanocyte lineage, as shown by the above mentioned protein atlas link and the breast cancer- and glioma references that we discuss in the Discussion section. However, we do not intend to identify exclusive melanoma markers. Instead, we aim at increasing our understanding of this disease by identifying proteins that are not or weakly expressed in the untransformed state and increase in level in the transformed state, where they help maintaining tumor features. These criteria are fulfilled by FOSL1, as shown in figures 3 and 5. Established melanoma oncogenes or important pathways (such as BRAF, NRAS, PTEN/AKT pathway, Wnt/beta catenin) are also not exclusive for melanoma, but their investigation has greatly improved our knowledge about the disease. As FOSL1 is a direct target of the MAPK pathway, which is activated in basically all kinds of melanoma, we consider it an important factor to investigate more closely in the future.

“3. Is there any connection between FOSL1 overexpression and B-Raf/N-ras mutation?”

- In the first version of this manuscript, we only addressed this question in the Discussion part, citing the publication by Packer et al., PCMR, 2009. The authors found a decrease of FOSL1 after pharmacological and siRNA-mediated inhibition of the MAPK pathway (3rd paragraph of the Discussion section). To investigate this question in more detail, we have now performed a FOSL1 Western blot of
1) unstimulated HERmrk cells, EGF-stimulated HERmrk cells, and EGF-stimulated HERmrk cells in presence of the MEK inhibitor U0126 (our control)

2) two human melanoma cell lines with or without U0126 treatment. Both melanoma cell lines possess an endogenous MAPK pathway activation, which is either due to the BRAF^{V600E} mutation (A375) or due to the NRAS^{Q61K} mutation (Mel Juso). In both cell lines, MEK inhibition strongly decreased the FOSL1 protein level (this is described in the new Figure 5A and in the last paragraph of the Results section).

The data suggest a link between the respective mutations in the RAS/RAF/MAPK pathway and the expression of FOSL1.

We have marked the major text changes by underlining the respective section.

We hope that with this new version of the manuscript we could rebut the doubts of the editor and the reviewers and ask you for the favour to consider our manuscript for publication in BMC Cancer.

Sincerely,

Svenja Meierjohann