Author's response to reviews

Title: Suppression subtractive hybridization profiles of radial growth phase and metastatic melanoma cell lines reveal novel potential targets

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Author's response to reviews: see over
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Melissa Norton
Editor-in-chief of the BMC Cancer

Subject: MS: 1758445937152252

Dear Dr Norton,

I am pleased to enclose here the revised form of our manuscript “Suppression subtractive hybridization profiles of radial growth phase and metastatic melanoma cell lines reveal novel potential targets”, authored by Josane de Freitas Sousa and myself.

Background, Results (specially the first topic) and Discussion sections were reformulated to conform to the reviewers concerns and to include requested information. Also, we include here our response to the specific points raised by the reviewers.

Thank you for your consideration.

Sincerely yours,

Enilza M. Espreafico
Reply to reviewers:

Referee 1.

David Julian Easty

Major compulsory revisions:

1. **The use of one RGP melanoma WM1552C:** Our initial intention was indeed to use a pool of RGP melanoma cell lines. However, according to analyses done by several groups as well as our laboratory, WM1552C was the only cell line within a small group of 3 cell lines that we obtained from the Wistar Institute to meet the criteria of low aggressiveness. We included detailed information to respond to this concern in the first part of the Results section. Also, we rewrote part of the Background section to conform to the proposal and approaches used in our work.

2. **Sex of patient WM1552C cells:** The patient was male and this information was included in the legend of Fig. 4.

3. **Chromosome 1 in WM1552C cells:** A translocation involving 1p22 has been reported for this cell line, but extra copy of chromosome 1 was not observed. Also, we succeeded to find information for the metastatic cell line WM9 not involving chromosome 1 (chromosomes 6 and 7), and for VGP cell lines WM278 (paired with WM1617) and WM793 (paired with 1205Lu) involving chromosome 1, including loss of the long arm and a translocation, respectively. This information was included in the Discussion section, in the beginning of the topic *Potential metastasis suppressor pathways*.

Minor essential revisions:

1. **Further details of work by Haqq et al:** This was included in the Results section (within the topic *Analysis of the expression profile of the genes represented in the SSH libraries in a panel of melanocytic samples using a publicly available microarray study*). Some information can also be found in the Material and Methods.
Referee 2:

Keiran Smalley

Major compulsory revisions:

1. **Establishment of metastasis and stepwise acquisition of oncogenic mutations:** We reorganized the Background section to include the comment suggested here (third paragraph). We feel that this suggestion contributed to improve the text.

2. **Concern about the genes identified been simply genes that are not expressed in WM1552C cells.** This is a pertinent concern, but we argue that several lines of evidence implicate many of the genes identified here in invasion, metastasis and cell survival/proliferation. Interestingly, all validated genes from the Met library (MITF, PLP1 and HLA-DR) showed not only low expression in WM1552C, but clear upregulation in metastatic cell lines. In addition, a subset of genes confirmed in microarray studies the differential expression profile associated to melanoma progression. So, perhaps, although some genes may only reflect bystanders, the fact that the selected RGP cell line meets several criteria of low aggressiveness turned to be a positive influence for the detection of many genes relevant to melanoma progression.

Minor essential revisions:

1. **Spellings** - They were corrected in the text.

2. **Discussion shortening** – We agreed with this point and did some shortening and reformulation of the Discussion section. A point regarding selection of the cell lines was transferred to the Results section (first topic) and several points were shortened or removed. Some information that were requested by other reviewer (chromosome losses) or others that we judge relevant were complemented (for instance, a point made on WNT5A). We also moved the last paragraph to the Conclusions section to meet the requirement of the journal. Although, shortening was not substantial, we feel that this allowed for a significant improvement in the discussion.

Discretionary revisions:

1. **Immunohistochemistry** – I entirely agree with the reviewer, but we are not enrolled at a Pathology Department and collaborations are only beginning to be set up, but I am sure that in the near future we will be able to test several of the genes reported here for the protein expression profile of melanoma samples. This will be a necessary step to prove their usefulness as markers. Unfortunately, this is not possible for this paper.
Referee 3:

Mohammed Kashani-Sabet

General – The three progression markers - not the most important - Initially we thought to extend our analysis to other markers to assure better selection of the cell lines. However, heterogeneity in gene expression profiles linked to melanoma progression makes it difficult to select for several cell lines sharing common markers (mainly among less aggressive ones, which are more limited in number). Concerning the importance of these genes, at least regarding KISS1, it appears that the results obtained by Dissanayake et al JBC 282:17259, 2007 raises the possibility that downregulation of KISS1, in response to Wnt5a signaling, is somehow implicated in the process of epithelial to mesenchymal transition. Besides, these markers, functional parameters also accounted for the selection of this single cell line as the representative of low aggressiveness, as detailed in the Results section.

Major compulsory revisions:

1. Rationale to leave out VGP – The Background section and Topic 1 of Results were reformulated to respond to this question. We feel that this suggestion led to a great improvement.

2. The missing lanes in Fig. 3 – The figure legend was reformulated to include this information. They mean that the corresponding cell line was not included in the northern blot. They were introduced for better alignment of the northern blot panels. We hope that with this explanation we were able to clarify this point.

Minor essential revisions:

1. Spellings – We thank the reviewer for helping with the language problems. All requested corrections were made in the text and we revised the entire text.