Author’s response to reviews

Title: Role of intracellular and extracellular annexin A1 in migration and invasion of human pancreatic carcinoma cells

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Version: 2 Date: 31 October 2014

Author’s response to reviews: see over
Dear Dr. Petrella,

A pleasant day to you.

Your manuscript has now been peer reviewed and the comments are accessible in PDF format from the links below. Do let us know if you have any problems opening the files.

Referee 2:
http://www.biomedcentral.com/imedia/1675946351147306_comment.pdf

Referee 1:
http://www.biomedcentral.com/imedia/2699278701464173_comment.pdf

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 27 November 2014. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=1835388814133059. You will find more detailed instructions at the base of this email.

Please don’t hesitate to contact me if you have any problems or questions regarding your manuscript.

Warm regards,

Annie

Annie Lyn Bravo
on behalf of Prof Andreas Bikfalvi
Journal Editorial Office
BioMed Central
e-mail: editorial@biomedcentral.com
Web: http://www.biomedcentral.com
Dear Dr. Bravo,
Re: Revised manuscript No.: MS: 1835388814133059

Many thanks for your letters of October 28th

Please find here enclosed a manuscript entitled:

“Role of intracellular and extracellular annexin A1 in migration and invasion of human pancreatic carcinoma cells”

by Belvedere R. et al that we are submitting for publication in BMC Cancer

The manuscript has been revised according to Reviewers’ comments as follows.

REVIEWER’S REPORT #1

Title: Role of intracellular and extracellular annexin A1 in migration and invasion of human pancreatic carcinoma cells.

Version: 1Date: 17 October 2014

The study by Belvedere and colleagues investigates the potential role of ANXA1 in the progression of pancreatic carcinoma. They examined the expression and localization of ANXA1 in MIA PaCa-2, PANC-1, BxPC-3 and CAPAN-2 cells and then investigate the role of ANXA1 in PC cell migration and invasion. They found that a huge expression and a variable localization of ANXA1 in sub-cellular compartments of PC cells and that ANXA1 reduction inhibited MIA PaCa-2 and PANC-1 cell migration and invasiveness rate. These effects were associated with cytoskeleton remodelling and FPR activation. A cleaved form of ANXA1 (33kDa) was found in MIA PaCa-2 cells. ANXA1 can be detected in the supernatants of MIA PaCa-2 cells, which could increase the migration rate of PANC-1 cells. These findings will be of interest to researchers to exploring the underlying mechanism of ANXA1 in cancer progression. This paper is well written, but there are many concerns below.

Minor comments:
1. The pro-oncogenic role of ANXA1 be mediated by FPRs signalling have been widely discussed. However, ANXA1 cleavage and nuclear expression were not fully investigated. The author should discuss the possible mechanism(s) in the discussion section.

1. Author’s response:
We include in the discussion section short sentences about the possible mechanisms underlying ANXA1 cleavage and nuclear translocation as well as the possible functions of this ANXA1 forms in tumours (lines 515-523).

2. There are many minor errors in the paper. For example, Line 47: the expression should be “The expression…”; line 52: in all…should be “In all…”; figure 1A in line 329, 457 should be Figure 1…

2. Author’s response:

According to reviewer’s comment, we revised the manuscript to correct these errors (lines 47, 52, 328, 457).

3. In Figure 1 legend, what does “quantitative analysis of E-cadherin expression/vimentin expression” mean: mRNA or protein?

3. Author’s response:

In the Figure 1 legend we referred to the percentage of cells positive for E-cadherin and vimentin proteins analyzed by confocal microscopy. However, we add method information in the figure legend 1, lines from 842 to 843 to better clarify this point.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published.

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 # 2

Title: Role of intracellular and extracellular annexin A1 in migration and invasion of human pancreatic carcinoma cells.

Version:1 Date:24 October 2014

Major Compulsory revisions

Introduction:

1. The terms recurrence and metastasis are used interchangeably. Please be specific on your intention. Additionally there is no distinction made between carcinogenesis and progression, PC development and PC progression. Please clarify.

1. Author’s response:

We revised the manuscript according to reviewer’s comment (lines 83, 99, 131, 401, 485, 491).

2. Please include a hypothesis for the study. It is mentioned that “ANXA1 should be considered as a novel therapy”, This study is not designed to answer this question and can be mentioned in the discussion.
2. **Author’s response:**

   According to reviewer’s comment we move the study hypothesis from introduction to conclusions section (lines 585-588).

3. Can the data in Figures 1A, 2A and 2D be quantified to lend itself to statistical analysis?

3. **Author’s response:**

   According to reviewer’s comment we add quantification of protein bands in Figures 1A, 2A and 2D.

4. Please provide statistical analysis of figure 1C and 1D.

4. **Author’s response:**

   *E-cadherin and vimentin expression in PC cell lines is widely characterized. Therefore, in Figures 1C and 1D we reported for each cell line the absolute E-cadherin and vimentin expression just to confirm the effectiveness of our experimental model. No statistical comparison between cell lines was required. However, we add short sentences in Figure 1 legend to better clarify this point (lines 842-844).*

5. In the results section please elaborate on the statistical methods used for each result.

5. **Author’s response:**

   *Statistical analyses were elaborated according to reviewer’s comment in figure legends: Figure 1 legend lines 837-838, 842-844; Figure 2 legend lines 853-854, 856-857, 863-864; Figure 5 legend lines 889, 892-893, 897, 900-901; Figure 6 legend lines 908-909. No statistical comparison was made in Figures 3 and 4.*

6. Is there a dose response to ANXA1 in all the analyses performed? If so is there a minimum and maximum effective level?

6. **Author’s response:**

   *We performed an Ac2-26 dose-response curve (starting from 50 nM up to 10 µM) in all the performed analyses in order to chose the minimum efficient concentration (1 µM). Dose increment didn’t lead to augmented effects.*

7. What is the difference between the MIA PaCa2 and PANC1 cell lines to produce differential results in Figure 3 and 4?

7. **Author’s response:**

   *According to reviewer’s comment we include some information about Figure 3 concerns in the discussion section (lines 557-568). In Figure 4 no significant differences between MIA PaCa-2 and PANC-1 cell lines were showed.*

8. Does ANXA1 exist as splice variants?

8. **Author’s response:**

   *No evidences are reported regarding this point.*
9. Is there evidence to suggest that the secreted form of ANXA1 interacts with cellular receptors or is taken into cells to produce an effect?

9. **Author’s response:**

*Unfortunately, no experimental evidences are reported about the interaction of the secreted form of ANXA1 protein with receptors other than FPRs neither about ANXA1 cell uptake from extracellular environments.*

10. In figure 4, why was qualitative PCR used and not quantitative? Can both be provided?

10. **Author’s response:**

*While we used qualitative PCR to verify FPR expression in our PC cell lines (Figure 4 panel B), we performed the cytofluorimetric assay in order to confirm FPR-1 and-2 protein expression on MIA PaCa-2 and PANC-1 cell lines (Figure 4 panel A). This assay allows us to be confident in FPR effective membranes expression in PC cells.*

Discretionary

1. It would be interesting to measure the ANXA1 levels in patients with PC and correlate the progression and/or metastatic rate to ANXA1 expression.

2. It would raise the level of this manuscript if you could measure the ANXA1 cellular localization and level in the primary tumours and/or metastasis in patients with PC.

**Level of interest:** An article of importance in its field

**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:** I declare that I have no competing interests.

Yours sincerely

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