Author's response to reviews

Title: Tumor-induced loss of mural Connexin 43 gap junction activity promotes endothelial proliferation

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Author's response to reviews: see over
Dear Mr. Relox,

Thank you for opportunity to revise our manuscript, “Tumor-induced loss of mural Connexin 43 gap junction activity promotes endothelial proliferation” by Choudhary et al. We appreciate the reviewers’ minor comments and suggestions, which we have addressed point-by-point below. Additions and changes to the text are highlighted in yellow.

We hope that our revised manuscript is now acceptable for publication in *BMC Cancer*. We look forward to hearing your response soon.

Best regards,

Linda Metheny-Barlow  
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**Response to Reviewer 1:**

Minor essential revisions:

1. *Page 11, line 269 statistics:* We have changed the statistics designation in the figure legends to the statistical conventions as suggested by the reviewer for better consistency across figures; exact p values are still maintained in the text.

2. *Fig. 1D:* ‘CASMC’ corrected to ‘vSMC’.

3. *Fig. 2B legend:* ‘left panel’ removed.

4. *Please increase size of immunolocalization picture in Fig. 2C:* Size increased as requested.

5. *Quantification for Fig. 3C:* We have added quantification of the data in 3C as requested, with description of time course data in the text.

6. *Time point for Fig. 3D:* We have indicated that time point shown was 24h treatment.

7. *Fig. 5B legend unclear:* The legend in 5B has been reworded to make it clearer to understand.

**Response to Reviewer 2:**

Minor essential revision:

1. *Don’t see RT-PCR data as described:* Our quantitative RT-PCR data is shown in Fig. 3E; we have clarified the description of the normalization in “Methods”.


Discretionary:

*Formula for tumor volume calculation:* We have included the reference (Tomayko and Reynolds et al., 1989; new ref #16) which supports our choice of tumor volume calculation. The calculation we chose in our experience is more accurate for tumors which may grow with large differences in length compared to width (which we sometimes see with mammary fat pad tumors).

**Response to Reviewer 3:**

Minor essential revisions:

1. *Page 2, line 43 missing word:* word omission (‘cells’) corrected.
2. *Paragraph transition, Page 15/line 354:* The introductory sentence has been reworded to improve clarity of rationale.
3. *Page 16, line 361:* We have revised the statement for better accuracy and clarity.
4. *Missing abbreviations:* Additional abbreviations used in the text (including NG2) have been added.
5. *Differences in Metastasis:* Interestingly, while we do not see changes in primary tumor growth, we do observe increases in the incidence of lung metastasis in Cx43 heterozygous mice. We feel, however, that this is out of the scope of the current manuscript and is being characterized for future publication.
6. *Significance of Cx changes in endothelial cells:* While some experiments do show slight increase in Cx43 in HUVEC and HBMEC in response to tumor conditioned media, the changes are not statistically significant (HUVEC, p=.399, n=3; HBMEC, p=.122, n=3).
7. *Fig. 1D label:* Corrected to ‘vSMC’.
8. *Description of Quantification in Fig. 2D:* We have added a more detailed description of the confocal quantification in the “Methods”.
9. *Please show statistical significance for Fig. 3D:* Statistical significance has been added as requested.
10. *Changes to Figure 4:* We have revised the labels in Panels A and C to indicate which cell type was used in each panel. In Panel B, the relative levels of Cx40 (0.8 at 24h, 1.2 at 48h) and Cx45 (0.9 at both 24h and 48h) are only slightly altered, which did not reproduce. Panel E: We respectfully believe that the inhibitor data in Panel E should be included as part of Figure 4, as it clarifies that the growth inhibitory effects of mural cell Cx43 on endothelial cells in co-culture requires functional Cx43 gap junctions, and cannot be attributed to the gap junction-independent growth inhibitory functions that have been described for Cx43 in some cell types.
11. *Please show immunoblot for Cx43 restoration in Fig. 5:* Immunoblot added as Fig. 5C as requested.