Reviewer's report

Title: Increase of Gap Junction Activities in SW480 Human Colorectal Cancer Cells

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Reviewer: jerome gilleron

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Major Compulsory Revisions

In this work entitled “Increase of Gap junction Activities in SW480 Human Colorectal Cancer cells”, Bigelow and Nguyen, try to decipher the impact of the chemical PQ1 on colorectal cancer cell line gap junction functionality. If the experiments are well done and the results provide mechanistic insight on PQ1 actions on Cx43-based gap junction functionality, the overall interest of this study is very limited. Indeed, in the figure 2 the authors clearly evidenced that overexpressing Cx43 leads to an increase of gap junction intercellular communication, but still is not impacting either on cell proliferation or on cell survival.

Specific comments:

1. The authors should add the citations referring to the intracellular localization of Cx43 phosphorylated isoforms since this information is important.

2. The scrape loading assay is not the more sensitive assay used today to determine the functionality of gap junction, but it should be sensitive enough to support or invalidate the hypothesis of the authors. However, essential control using AGA, oleamide or Carbenoxolone seems required to determine the transfer distance after fully blocking GJIC. Indeed, if TPA is disturbing Gap junction, it is not per se a GJIC functional inhibitor.

3. In discussing the results of the figure 4, the authors concluded that the findings suggest that PQ1 act on existing Cx43 and not on expression. The authors should show by imaging an increase of Cx43 staining at plasma membrane. This result is important and easy to obtain.

4. Based on the lack of stars, it seems that the results plotted on the figure 5 is not significant, why the authors conclude that increase of gap junction functionality by PQ1 may act on Akt activation? First this could be correlative and not causal, second the data don’t support the hypothesis since not significant.

5. Same question for the figure 6. Indeed if here the effect is significant, how the author could exclude that PQ1 acts on MAPK activation independently to GJ? Inhibiting GJIC could solve this problem and demonstrate causality.

In conclusion, this work was well conducted, even if missing controls are required. Nonetheless, the interest of this study is strongly diminished by the fact
that the authors demonstrates that overexpressing Cx43 in SW480 cells does not impact on cancer cell proliferation inhibition or survival. For these reason I do not recommend this paper for publication in BMC cancer in the present form.

**Level of interest:** An article of limited interest

**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