Reviewer’s report

Title: Cytotoxicity of amide-linked local anesthetics on melanoma cells via inhibition of Ras and RhoA signaling independent of sodium channel blockade

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Reviewer: Ru Li

Reviewer's report:

This manuscript analyzed the cytotoxicity of ropivacaine, lidocaine, and bupivacaine on melanoma cell lines. The tested cell functions included transwell migration, proliferation, and apoptosis. Ropivacaine and lidocaine but not bupivacaine were found to inhibit cell migration and proliferation and induced apoptosis in melanoma cells at millimolar concentration. Those effects of ropivacaine on cell functions are independent on its effect on sodium channel. The authors further identified RhoA and Ras as the targets of ropivacaine induced inhibitory effects on cellular growth and migration, respectively. The data supported the above-mentioned conclusion. However, there are some fundamental issues associated with the experimental design, which need to be clarified before further consideration.

1. Commonly used amide-linked local anesthetics in clinic including lidocaine, mepivacaine, ropivacaine, bupivacaine, and levobupivacaine. Please justify the choice of lidocaine, ropivacaine and bupivacaine out of other local anesthetics.

2. As the authors discussed that the mean peak plasma concentrations of local anesthetics following transversus abdominis plan block is between 1 to 3 µM, while the concentrations used in the study are between 0.25 to 2 mM, which are more than 1000 times higher than the clinical concentration. Please justify the relevance of those results to clinical setting. There is a paper published in 2018 by Li et al on BMC Cancer, "Effect of local anesthetics on breast cancer cell viability and migration", in which clinical relevance doses of local anesthetics (0.02 to 0.1 mM) were analyzed on breast cancer cells. Please consider include a couple of doses to that end.

3. For transwell migration assay, what is the size of pore on the insert membrane? For Annexin V staining, please show representative image of flow cytometry for each treatment? For both proliferation and apoptosis assay, please justify 72-hour treatment time? Why not 24 or 48 hours? What is the doubling time for both cell lines?

4. Ropivacaine and lidocaine enhance the inhibitory effect of vemurafenib and dacarbazine. Was the inhibitory effect synergistic or additive?

5. How dose lidocaine affect GTPase activities?

6. In the presence of sodium channel inhibitors, dose ropivacaine still affect RhoA and Ras activities?
7. Typo on page 8 line 1 and line 5 (Fig. 3D to F) not (Fig. 2D to F). Also some grammar errors in the body text.

8. Statistical analysis are not clear. What software was used for statistical analysis? Method section mentioned the use of standard deviation (SD). However, figure legends all stated mean +/- SEM, which is totally different from SD. Also different statistical analysis method should be chose according to different type of bar chart, which did not mention anywhere in figure legend and the "one-way ANOVA or t-test" in method section is ambiguous.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Needs some language corrections before being published

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