Author's response to reviews

Title: Expression of inwardly rectifying potassium channels (GIRKs) and beta-adrenergic regulation of breast cancer cell lines.

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Author's response to reviews: see over
Dear Sir or Madam:

We are submitting the revised manuscript for possible publication in BMC Cancer entitled “Expression of inwardly rectifying potassium channels (GIRKs) and beta-adrenergic regulation of breast cancer cell lines.” Response to reviewer’s comments is as follows:

Reviewer: Satya Narayan

1) Explanation for lack of GIRK3 studies:

According to a review article (Yamada et al., Pharmacological Reviews, 50: 723-757, 1998), reviewed during the research, we felt it was unlikely to see GIRK3 expression since it is found almost exclusively in the brain. A PubMed search Nov. 8, 2004 failed to indicate expression of GIRK3 other than in the brain. In addition, one of the main functions of GIRK3 (found during the PubMed search) is inhibiting plasma membrane expression of the other GIRK channels making it unlikely to be involved in the observed responses (Ma et al., Neuron, 33:715-729, 2002). However, we have added at the end of the discussion the following statement:

**Future studies are also needed to determine if GIRK3 is involved in breast cancer. However, we think this unlikely because one of the functions of GIRK3 is to inhibit plasma membrane expression of other GIRK subunits [36].**

We also added reference 36 to the References.
2) “There is an interesting observation between expression levels of GIRK1 and cyclophylin. The cell line expressing more GIRK1 expressed low levels of cyclophylin.” This type of observation is not possible with these experiments. The following statement was added to the Figure legend for both Figure 1 and 2.

**PCR reactions resolved on this gel were in the plateau phase of PCR, therefore concentrations of PCR amplified cDNA samples cannot be compared.**

“The level of cyclophylin needed to be included with samples for GIRK4 RT-PCR.” This information was found in the text, however, the following statement was added to the figure legend for Figure 2 to make it more clear:

**Cyclophylin was used as a positive control for both GIRK2 and 4.**

3. “The results in Figure 3 should be quantitated and normalized with 18S for a clear presentation.” In the submitted Figure Legend for Figure 3 it stated:

**Densitometry Girk 1/ Densitometry 18S-control-0.5494 ± 0.0285 (SD); propranolol treated 0.9028 ± 0.0348 (SD), p < 0.0001.**

This has been rewritten to make it more clear:

**Densitometry values of the bands for Girk 1 were normalized by the densitometry values of the bands for the 18S primers/competimers. Normalized values for control were 0.5494 ± 0.0285 (SD); and normalized values for propranolol treated were 0.9028 ± 0.0348 (SD), p < 0.0001.**
Reviewer: Naseema Hoosein

1) ‘It is unclear in the abstract (page 2, line 6 from bottom) for how many days the 30 min daily treatment was done.” The words “for 7 days” were added.

2) “page 9, start of last paragraph would read better if changed to “To determine if GIRK channels are….” This change was made.

3) “page 13, in the conclusions…it is unclear the effect of beta-adrenergic antagonists on potassium flux, if any, is unclear.” In the sentence “Beta-adrenergic antagonists have both long term effects on gene expression and beta-adrenergic agonists and antagonists have short term effect on potassium flux and cellular signaling pathways.” The words “and antagonists” were removed.

In addition, we corrected a typo found in the Figure 7 Figure legend. Unnecessary capitalization in subsection headings was corrected, and the heading “Figures” was changed to “Figure legends”.

Thank you,

Howard K. Plummer III