Author's response to reviews

Title: Anti-oncogenic and Pro-differentiation Effects of Clorgyline, a Monoamine oxidase A Inhibitor, on High Grade Prostate Cancer Cells

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Author's response to reviews: see over
July 10th, 2009

RE: MS: 4810759952137743

Dear Dr. Edmunds,

Thank you for the thoughtful comments on our revised manuscript entitled "Anti-oncogenic and Pro-differentiation Effects of Inhibition of Monoamine oxidase A on High Grade Prostate Cancer Cells" (MS: 4810759952137743). We have revised it based on the critiques of the reviewers in a point-by-point manner. A detailed explanation of the revisions with an indication of where the changes have been made in the text is attached.

Thank you for your consideration of publishing our manuscript. Please let me know if there is anything else we can do to improve it.

Sincerely,

Donna M. Peehl, Ph.D.
Response to comments by Dr. Audrey Player:

MINOR:

1. page 7, In the "METHODS-cDNA Microarray" section... Statement was "cDNA probes were prepared from 50-70mg "? Please confirm as this seems extraordinarily high. Normally microgram quantities are applied to the array. the concentrations refer to the total amount used for the entire study?

It was a typo. The mistake was corrected to 50-70 µg. This change was highlighted on page 7 line 2.

2. page 8 In the "METHODS-Quantitative Real time " section... the authors note "Transcript levels of GAPDH were assayed simultaneously with each of the following ten genes as an internal control to normalize transcript levels in control and treated cells". More genes were included after the revision.

There were total 20 genes validated. The correct number was used to replace the initial number. This change was highlighted on page 8 line 11.

3. page 16 " Clorgyline induces differentiation...." Address / note , briefly, the fact that EZH2 is not observed as differentially expressed on the microarray? here or in discussion.

We looked at EZH2 and ADRB2 expression in our microarray data. ADRB2 was upregulated by clorgyline (2.1 and 1.7 fold at 6 and 24 hr, respectively; data for 96 hr was not available after filtering), and EZH2 expression showed minimal increase (1.0, 1.2, and 1.2 fold at 6, 24, and 96 hr, respectively). This result was described in Results section on page 15 line 3.

4. page 17-18. " Validation of the effects of clorgyline using E-CA-90" ...EZH2 and other Polycomb genes represented (i.e., differentially expressed) in the E-CA-90? Please address in the text.

We examined the expression of EZH2 and the four Polycomb signature genes, MYO6, SATB2, SOCS2, and RGC32, by qRT-PCR in E-CA-90 cells. Similar to E-CA-88 cells, expression of three of the four genes was significantly upregulated in treated E-CA-90 cells compared to control. Specifically, EZH2 was downregulated by 40% and MYO6, SOCS2, and STAB2 were increased by 3.6, 3.7, and 2.6-fold, respectively. These results suggest that clorgyline induced genes suppressed by the Polycomb complex in E-CA-90 cells. These results were described on page 16 from line 10.

DISCRETIONARY:

1. page 8 " METHODS- Quantitative Real time..."..Chart of primers to make it easier for the readers, possibly supplemental. Currently primers are listed in the text. there are many and its a bit hard to follow.

The primer sequences were listed in Additional file 1, and this change was highlighted on page 8 line 12. The corresponding legend was provided on page 35 line 2.