December 1, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3329-edited.docx).

Title: Autophagy Inhibition by Chloroquine Sensitizes HT-29 Colorectal Cancer Cells to Concurrent Chemoradiation

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The manuscript has been improved according to the suggestions of reviewers:

1 No language certificate has been submitted, as all authors are native English speakers.

2 Author contributions have been added to the title page, page 1.

3 An abstract has been added to page 3-4 in accordance with the minimum word counts.

4 A Core Tip description was added and can be found on page 4.

5 Revision has been made according to the suggestions of the reviewer

1. *The experimental design involves 3 factors: Cell lines (2 levels), treatment groups (8 levels), measurement time points (6 time points). Within cell lines, 3 concentrations were used. For RT, 3 active dose levels were used. This brings a total of more than 570 reads for one outcome measurement at different combinations of these factors (levels). The experiment included 7 outcome measurements. For each of the outcome measurement, authors didn't present the whole picture of the data. It seems like authors only reported selected combination(s) data for each measurement. This is suspicious to me that authors were selecting the results and only report the ones that show "positive results" in the manuscript. Without seeing the whole picture of the data, or number of multiple comparisons, it is hard to distinguish whether the results presented in the manuscript is just by chance, or it is real. Please clarify.*

   Due to the number of variables needed to address our central question (i.e. cell lines, drug concentration, radiation dose, time points), we first started our experiments using the MTT assay. This allowed us to look at cell viability for 80+ combinations of drug dosages for each radiation treatment. Once we established the drug concentrations that worked best in synergy from the MTT assay, we applied them throughout our remaining experiments.

   Both positive and negative data were described throughout the manuscript. For example in figure 3B, the HCT116 cell lines did not show radiosensitizing effects with chloroquine on clonogenic

assays while the HT-29 cells did. As a result, the additional data in figure 3C, D and E looking at possible mechanisms for sensitization or increased cell death was only displayed for the HT-29 cell lines since this line was the one with sensitivity. We believe we represented to data fairly, stating both positive and negative findings. Furthermore our discussion and conclusions ddi not overstate our findings and emphasizes that radiosensitization was found only in the HT-29 cell line.

2. *Authors didn't indicate how many replicates were conducted. This brings question of whether the statistical analysis is appropriately conducted.*
All experiments were performed in triplicate in three independent experiments and this has been further clarified in the methods section on page 10 of the manuscript under the Statistical Methods section heading. In performing triplicate experiments this allows for a mean to be calculated and compared between treatment groups, ensuring appropriate statistical analysis.

3. *Not all outcome measures are continuous (not to mention the normality assumption), for example, apoptotic -- binary, colony forming assays -- binary. Student's test won't be appropriate for these measures. Further, based on the design described in the text, this is really a ANOVA type of analysis for the normally distributed measures, and multivariate logistic regression type of analysis for binary measures.*
All of the final outcomes of our experiments provided data that was continuous: (1) Fluorescence Microscopy quantified the number of positive cells per 50 cells resulting in a mean percentage compared across groups; (2) Colony Forming Assays calculated survival fractions by dividing the number of colonies counted per plate by the number of cells plated taking into account the plating efficiency resulting in a mean survival fraction for each treatment group; (3) Cell Cycle Arrest and Apoptosis experiments produced data represented by a percentage of cells in each phase of the cell cycle or a percentage of cells that expressed the apoptosis markers and the percentage numbers were compared between groups. For all of these continuous data, the experiments were performed in triplicate so a mean and standard deviation could be calculated and compared between treatment groups. An ANOVA analysis is used for normally distributed data when you want to compare more than two means. This analysis reduces the type I error that can occur by too many direct student t test comparisons. When an ANOVA test is significant it indicates that one of the means is significantly different from the others, but does not tell you specifically which group is significantly different from the others. A student t test for normally distributed data or a Mann Whitney test for data not normally distributed can give you the actual p-value when comparing between two groups for significance. That is why the Students' t test was used to determine p-values in our experiments. We believe these are the correct p-values for statistical significance as the data is presented.

6 The Comments section is complete and can be found on page 17 of the edited manuscript.

7 References and typesetting were corrected.

8 The figures have been put into a word document.

Thank you again for considering our manuscript for publication in the *World Journal of Gastroenterology*.

Sincerely,

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