Peer Review File

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Review Comments:

It is a reasonable manuscript for this journal. Overall, it is written sloppily without attention to detail, which gives me a negative connotation. However, if that is fixed, would be reasonable to accept.

The article submitted by Li et al., studies the radioresistance effect of IGF-1R in colorectal cancer. The authors reported correlation between IGF-1R mRNA and protein levels and the radioresistance using patient tissues and colorectal cancer cell lines. Using siRNA and chemical inhibitor against IGF-1R, they describe a mechanism responsible for the radioresistance. Together, the results convincingly support the hypothesis. The experiments seems rigorously done and this article will be a nice addition to the literature, however many issues, mostly minor should be addressed for publication.

Minor reviews:

- Please, keep consistency for writing "IGF-1R" in the manuscript. The correct annotation would be IGF1R for gene nomenclature and IGF1R for the protein.
- In the abstract is mentioned the SW620 cell line. However it seems that all experiments were done using SW480 cell line. Please correct accordingly
- In the results section of the abstract, please change "rectal cancer" by "colorectal cancer" since all cell lines used were isolated from colon and not rectum.
- In the end of 2nd paragraph of introduction, the sentence starting by "Ionizing radiation can increase…" is very confusing. Please modify it to improve the comprehension.
- In the material and methods, the 2.9 cell cycle assay description is very confusing. There are no results of Annexin V/PE in the manuscript and this technique cannot be used for a cell cycle analysis. Please re-write this section or redo the experiment.
- In the table 1, it would be a great to compare Radioresistant and Radiosensitive with their IGF1R level and show the Chi square value.
- In the results section 3.1, please define what is CA199 and CEA in the manuscript. In the corresponding figure (Fig 1.C) please indicate the R2 and the p value on the graph.
- In the results section 3.2, the authors wrote "…IGF1R would be positively correlated with radiation sensitivity…". According the results presented, the IGF1R is positively correlated with radiation resistance. Please change.
- In the figure 3.A and 3.D the Y-axis is "Relative mRHA" please change for "Relative mRNA" or "Relative expression". For the cell cycle analysis panels, it is not clear why there are no differences between 0Gy and 2Gy. Instead of showing the results as histogram, please provide a representative diagram (curve graph provided by the flow cytometer) for each condition. It will be more informative for the reader.
- in the results section 3.4 the authors wrote "Cell growth curve shows, compared…".

Please change it by “Crystal violet staining showed…” or anything else that does not mention “ cell growth curve” since there is no curve in figure 4.E
- In the result section 3.5, please mention either in the figure itself, figure legend or main text what doses of irradiation were used.
- In the figure 5.A and 5.B, the upper panel of the western blots seem to be wrong. The last row should be +/+ instead of +/-. In figure 5.C and 5.D please change “r-H2AX” by “γ-H2AX” or “gamma-H2AX” (then, change accordingly in the manuscript).
- In the results section 3.5, at the beginning of the second paragraph, please change “To analyze the effect of BM-754807 on the repair” by “To analyze the effect of BM-754807 on double stand DNA damages”
- In the discussion, please remove the underline under radioresistance.
- In the discussion, the paragraph starting by “As is well known…” please remove this sentence. It is very confusing for the reader.
- In the discussion, please change the sentence starting by “After DNA damage in the cell, H2AX phosphorylates…” by “After DNA damage in the cell, H2AX is phosphorylated”.
- In the discussion, remove anything related to apoptosis (see below in major reviews).
- In the discussion, change any “inhibit DNA repair” by “increase DNA damages”. The results only show analysis of DNA damages, but there is no experiment looking at DNA repair protein or kinetic.

Major reviews:
- CONTROL
- The result section 3.4 and the associated Figure 4, need to be re-evaluated.
• The authors keep mentioning in the result section 3.4 about apoptosis. However, there are no experiment looking at apoptosis quantification. Only proliferation assay and colony formation assay were realized. The authors must do cell death experiment to conclude of an increase of apoptosis. However, the study could only be limited to proliferation and clonogenicity. If so, the authors need to remove every sentences related to cell death in the current manuscript.
• The manuscript mentions “BMS-754807 can inhibit proliferation, both in irradiated and unirradiated cells”. However the figure 4 A-D shows the the BM-754807 does not affect the unirradiated cells.
• Results of SW480 treated with BM-754807 + irradiation in Figure 4.B (OD of ~0.5) are not coherent with the ones reported in Figure 4.G (OD of ~0.3). Authors need to redo the experiment of clearly mentioning in the manuscript this difference of consistency.

Response to Major reviews

Comment 1: The authors keep mentioning in the result section 3.4 about apoptosis. However, there are no experiments looking at apoptosis quantification. Only a proliferation assay and a colony formation assay were realized. The authors must

perform a cell death experiment to conclude an increase of apoptosis. However, the study could only be limited to proliferation and clonogenicity. If so, the authors need to remove every sentence related to cell death in the current manuscript.

Reply 1: Thank you for your suggestion. In our study, we only performed proliferation and colony formation assays, therefore, we have removed the text related to cell death or apoptosis in the revised manuscript as advised.
Changes in the text:
1. we deleted “induced apoptosis” in the results section of the abstract (see Page 3, line 1).

2. We changed “Based on the clear changes in proliferation and apoptosis in BMS-754807 treated cells induced by 2 Gy of radiation” to “Based on the clear changes in proliferation of BMS-754807 treated cells induced by 2 Gy of radiation” (deleted “and apoptosis”) in the result section 3.4 (see Page 12, line 13).

3. We deleted “In addition, as shown in Figure 4F, treatment with BMS-754807 enhanced the apoptosis induced by 2 Gy of X-ray irradiation compared to the radiation-only control. Similar results were observed in HT-29 cells (Figure 4G,4H). ”in the result section 3.4 (see Page 12, line 16).

Comment 2: The manuscript mentions “BMS-754807 can inhibit proliferation, both in irradiated and unirradiated cells”. However the figure 4 A-D shows the the BM-754807 does not affect the unirradiated cells.

Reply 2: Thank you for your comments. We have re-preformed the proliferation assay. In the new results, we found that BMS-754807 does inhibit the proliferation of unirradiated cells. However, studies have reported that BMS-754807 can inhibit the proliferation of breast cancer and glioma cells. In fact, in our original experiment, we also observed the same result, therefore, we have checked our initial raw data and graphs obtained. We apologize for entering the wrong data when producing the graph with the GraphPad software, and causing discrepancy between the graph and our description. We have re-performed the proliferation experiment and selected the new experimental data when generating the new figure. The new results showed that the combination of drugs and radiation can further inhibit cell proliferation.

Changes in the text: We changed figure 4A-F. (see Figure 4);

Reference:
(1)Chakraborty A, Hatzis C, DiGiovanna MP.Co-targeting the HER and IGF/insulin receptor axis in breast cancer, with triple targeting with endocrine therapy for hormone-sensitive disease.Breast Cancer Res Treat. 2017 May;163(1):37-50.

(2)Halvorson KG, Barton KL, Schroeder K, et al.A high-throughput in vitro drug screen in a genetically engineered mouse model of diffuse intrinsic pontine glioma identifies BMS-754807 as a promising therapeutic agent.PLoS One. 2015 Mar 6;10(3):e0118926.

Comment 3: Results of SW480 treated with BM-754807 + irradiation in Figure 4.B (OD of ~0.5) are not coherent with the ones reported in Figure 4.G (OD of ~0.3). Authors need to redo the experiment of clearly mentioning in the manuscript this difference of consistency.
Reply 3: Thank you for your comment. We apologize since we had entered the wrong data when using GraphPad software to generate the graph. We have re-preformed the proliferation assay.
Changes in the text: We changed figure 4A-F. (see Figure 4A,B,C,D,E,F).

Response to Minor reviews

1. Please, keep consistency for writing "IGF-1R" in the manuscript. The correct annotation would be IGF1R for gene nomenclature and IGF1R for the protein.
   Reply 1: Thank you for your suggestion. We have replaced all "IGF-1R" in the full text with IGF1R. (Totally 83 locations, including graphs)

2. In the abstract is mentioned the SW620 cell line. However it seems that all experiments were done using SW480 cell line. Please correct accordingly
   Reply 2: Thank you for your comment. We have replaced "SW620 cell line" in the abstract with "SW480 cell line". (see Page 2, line 12)

3. In the results section of the abstract, please change "rectal cancer" by "colorectal cancer" since all cell lines used were isolated from colon and not rectum.
   Reply 3: Thank you for your comment. We have replaced "rectal cancer" in the abstract with "colorectal cancer". (see Page 2, line 20)

4. In the end of 2nd paragraph of introduction, the sentence starting by "Ionizing radiation can increase…" is very confusing. Please modify it to improve the comprehension.
   Reply 4: Thank you for your suggestion and we apologize for the lack of clarity. We have rewritten this sentence for better clarity. (see Page 4, line 20-21)

5. In the material and methods, the 2.9 cell cycle assay description is very confusing. There are no results of Annexin V/PE in the manuscript and this technique cannot be used for a cell cycle analysis. Please re-write this section or redo the experiment.

Reply 5: Thank you for your suggestion. We have rechecked our original data. We had only conducted cell cycle experiments with PI staining. Therefore, we have changed the inaccurate description in the Methods section of the revised manuscript. We have also reanalyzed the data and produced a cell cycle diagram. (see Page 9, line 6-12)

6. In the table 1, it would be a great to compare Radioresistant and Radiosensitive with their IGF1R level and show the Chi square value.
   Reply 6: According to your suggestion, we have reanalyzed the data, calculated the Chi square value, and produced a new graph. (see Table 1)

7. In the results section 3.1, please define what is CA199 and CEA in the manuscript. In the corresponding figure (Fig 1.C) please indicate the R2 and the p value on the graph.
   Reply 7: In accordance with your suggestion, we have added the definitions and functions of CA199 and CEA in the manuscript. R2 and p values are presented in the corresponding graphs. (see Page 10, line 8-9; see Page 13, line 21; see Figure 1 C,D)

8. In the results section 3.2, the authors wrote "…IGF1R would be positively correlated with radiation sensitivity…". According the results presented, the IGF1R is positively correlated with radiation resistance. Please change.
   Reply 8: We apologize for the confusion, and have made the relevant changes to the text. We have replaced "sensitivity" in the abstract with "resistance". (see Page 10, line 21)

9. In the figure 3.A and 3.D the Y-axis is "Relative mRHA" please change for "Relative mRNA" or "Relative expression". For the cell cycle analysis panels, it is not clear why there are no differences between 0Gy and 2Gy. Instead of showing the results as histogram, please provide a representative diagram (curve graph provided by the flow cytometer) for each condition. It will be more informative for the reader.
   Reply 9: Thank you for your comment. We have replaced "Relative mRHA" in the abstract with "Relative mRNA". (see figure 3 A ,B). For the cell cycle analysis panels, we have, accordingly, provided a representative diagram (curve graph obtained using a flow cytometer) for each condition. (see figure 3 C).

10. in the results section 3.4 the authors wrote "Cell growth curve shows, compared…". Please change it by "Crystal violet staining showed…" or anything else that does not mention " cell growth curve" since there is no curve in figure 4.E
    Reply 10: We have replaced "Cell growth curve shows, compared…" in the results section with "Crystal violet staining showed…". (see Page 12, line 16).

11. In the result section 3.5, please mention either in the figure itself, figure legend or main text what doses of irradiation were used.
Reply 11: The irradiation doses are mentioned in the main text. (see Page 13, line 1)

12. In the figure 5.A and 5.B, the upper panel of the western blots seem to be wrong. The last row should be +/+ instead of +/-. In figure 5.C and 5.D please change "r-H2AX" by "γ-H2AX" or "gamma-H2AX" (then, change accordingly in the manuscript).
Reply 12: We changed +/- to +/+ in the figure(see Figure 5 A,B).All "r-H2AX" in the revised text and figures have been replaced with "γ-H2AX".(see Page 13, line 6, see Figure 5 C,D)

13. In the results section 3.5, at the beginning of the second paragraph, please change "To analyze the effect of BM-754807 on the repair" by "To analyze the effect of BM-754807 on double stand DNA damages"
Reply 13: We have replaced "To analyze the effect of BM-754807 on the repair" with "To analyze the effect of BM-754807 on double stand DNA damage". (see Page 13, line 5)

14. In the discussion, please remove the underline under radioresistance.
Reply 14: We have removed the underline under radioresistance. (see Page 14, line 12)

15. In the discussion, the paragraph starting by "As is well known…" please remove this sentence. It is very confusing for the reader.
Reply 15: We have removed the sentence starting with "As is well known…". For better clarity, we have revised the text to reduce ambiguity. (see Page 15, line 6)

16. In the discussion, please change the sentence starting by "After DNA damage in the cell, H2AX phosphorylates…" by "After DNA damage in the cell, H2AX is phosphorylated".
Reply 16: We have replaced "After DNA damage in the cell, H2AX phosphorylates" with "After DNA damage in the cell, H2AX is phosphorylated". (see Page 15, line 12)

17. In the discussion, remove anything related to apoptosis (see below in major reviews).
Reply 17: We have removed all text related to apoptosis from the revised manuscript. (see reply to the major comments).

18. In the discussion, change any “inhibit DNA repair” by “increase DNA damages”. The results only show analysis of DNA damages, but there is no experiment looking at DNA repair protein or kinetic.
Reply 18: We have replaced all “inhibit DNA repair” with “increase DNA damage”. (see Page 3, line 1; see Page 4, line 20; see Page 13, line 5; see Page 15, line 17; see Page 16, line 6;)