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OPEN PEER REVIEW

Effect of 5'-fluoro-2'-deoxycytidine, 5-azacytidine, and 5-aza-2'–deoxycytidine on DNA Methyltransferase 1, CIP/KIP Family, and INK4a/ARF in Colon Cancer HCT-116 Cell Line

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

3 comments · 16 Mar 2021 – 31 Mar 2021

Referee 1 Reviewer

MINOR REVISION 16 Mar 2021

The authors present a well-designed study that I recommend for publication.
I have no major issues, but I have the following comments:

- There were only a few grammatical errors that were highlighted.
- Keywords must be selected based on the MeSH system.
- In Materials and Methods: the study design should be added.
- Results: In Figure 2: which one is the control? The 24h, 48h, and control cases were not identified.
In Figure 3A: at the top of the figure, is the treatment duration 2h or 24h? Please edit it.
In Figure4: A and B; in order to demonstrate the differences between 24h and 48h, it is recommended to reset the vertical axis (Y) to the same scale.

- In the discussion, it is recommended to compare your results with more relevant studies.
Refer to the attached file.

Referee 2 Reviewer

MINOR REVISION 23 Mar 2021

Dear Editor
Many thanks for your kind email inviting me to review a manuscript entitled " Investigation of the effect of 5-azacytidine, 5-aza-2'–deoxycytidine, and 5'-fluoro-2'-deoxycytidine on INK4a/ARF family, CIP/KIP family and DNA methyltransferase 1 gene expression, cell growth inhibition, and apoptosis induction in colon cancer HCT-116 cell line".
In my opinion, this manuscript is acceptable for publication in your valuable journal, but there are some comments that must be addressed before publication.
You can find the comments in the attached file.

Associate Editor

MAJOR REVISION 31 Mar 2021

Comments to the authors:
1- The English of the manuscript needs to be revised.
2- The authors mentioned that they had conducted the previous studies. They should add references to the previous studies in the Introduction section.
3- The materials and kits used should be explained carefully in the materials and methods section. In the manuscript, only references to other articles were provided for them.
4- The authors should state the criteria for selecting concentrations in the present study.
5- qRT-PCR should be fully explained. The authors only mentioned that it is similar to that used in the previous studies.
6- The authors should explain what method they used to analyze the qPCR results (Livak, pfaffl or other methods).
7- The authors should explain whether they used DNase I digestion. Did they design the primers as exon junction primers?
8- The authors should explain what concentration was used in Figures 2, 3 and 4.

9- The titles of the tables were not informative.
10- The results obtained were explained briefly in the results section. They should be reported fully. For example, the authors should state what concentration was determined as IC50 in the present study. The other data should also be explained.
11- The Discussion should be revised. The authors should compare the results obtained with those of the previous studies.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

The authors did not provide a point-by-point response to the reviewers' comments in this round.

2 Revision 1

1 comment · 17 Jul 2021

Associate Editor

MINOR REVISION 17 Jul 2021

Comments to the authors:
1- The English of the manuscript needs to be revised by a person familiar with English.
2- Some sentences did not have any references. Every sentence in the manuscript should have a reference. The authors' findings are exceptions.
3- The results obtained from gene expression should be explained in more detail. It should be stated which genes showed decreased expression and which genes showed increased expression.
4- The title of Table 1 was very long. It should be shortened.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

The authors did not provide a point-by-point response to the reviewers' comments in this round.

3 Revision 2

0 comments

No public comments in this round.

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear editor

Thank you so much for your valuable comments. We responded to the comment carefully.

Best regards

Comment no.	comment	Response
1	Dear author, The P-Value reported in Table 1 does not indicate which groups were compared. In this regard, please describe it as a table footnote or change the layout of the table to indicate the P-Value Gives.	The below statement was added to the figure legend of table 1. It should be noted that the p-value indicates the difference between the control group (untreated cell line) with each experimental group treated with the compound mentioned in table 1.

4 Revision 3

0 comments

No public comments in this round.