Reviewer's report

Title: Knockdown of autophagy-related protein 5, ATG5, decreases oxidative stress and has an opposing effect on camptothecin-induced cytotoxicity in osteosarcoma cells.

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Reviewer: Stefania Meschini

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General Comments.

The paper by Hollomon M.G. et al. reports the results by a wide experimental work aimed to demonstrate that autophagy inhibition (ATG5 knockdown) has an opposite effect on the cytotoxicity induced by Camptothecin in murine metastatic osteosarcoma cell lines (DLM8, K7M3).

The use of two different osteosarcoma cell lines is justified because the K7M3 cells have a high basal autophagy compared to DLM8 cells. This difference results in a different response of the cells to the inhibition of autophagy and a different response to CPT treatments. The autophagy inhibition reduces cell death in the DLM8 cell line with low basal level of autophagy while increases cell death in K7M3 cells with higher basal autophagy. The inhibition of autophagy by ATG5 gene silencing reduces basal and CPT oxidative stress in DLM8 cells. The authors conclude that the inhibition of autophagy by ATG5 gene silencing protects of osteosarcoma DLM8 cells from CPT cell death.

In this work the authors conclude that the autophagy modulation (particularly inhibition) may have an effect on the chemotherapeutic response in cancer cells. Certainly the data of the effect of CPT on osteosarcoma cell lines is new, but it is not the fact that the CPT by inducing autophagy inhibition increases the effectiveness of chemotherapy ... see the work listed below:


The inhibition of autophagy sensitises colon cancer cells with wild-type p53 but not mutant p53 to topotecan treatment.

Li DD, Sun T, Wu XQ, Chen SP, Deng R, Jiang S, Feng GK, Pan JX, Zhang XS, Zeng YX, Zhu XF.

Although the discussion is well balanced the experimental part needs a careful review.

In conclusion, in my opinion, this paper must respond to the major compulsory revisions.

Specific points:

The materials and methods are appropriate and adequately described but the figures are missing key parts:
Figure 1A:
Insert a table that supports cell viability in which are included the total cells values, the percentage of live cells (TB negative) and dead cells (positive TB). This request is made to verify whether the treatment induces mortality or slowdown;
Flow cytometric analysis in this paper is accurate, but changes are necessary to clarify the results:
Figure 3A:
Specify the parameters in x-axis and y-axis;
Figure 4B: Specify the parameters in x-axis and y-axis;
Figure 4C and 4D Specify the cell lines which are discussed;
Figure 6A: Eliminate the written + TMRE, seen that this dye is used for the determination of all samples;
Caption of Figure 6:
1. CPT induces cell arrest and .... where are the data of the cell cycle??
2. Because the mitochondrial membrane potential was made to 24h when all other experiments in 48h, specify?
Experimental part of western blotting:
1. Specify the molecular weights beside the proteins and determine the bands quantization relate to the control
2. Specify the exact concentrations of the drugs used and no low ... medium... .
3. Figure 3C: is necessary to put LC31 protein expression to demonstrate the conversion of the molecule from the form 1 (indicating basal level of autophagy ) to 2 and specify the difference in molecular weight (18 kDa, 16kDa)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I DECLARE THAT I HAVE NO COMPETING INTERESTS