Reviewer’s report

Title: Ceramide Targets xIAP and cIAP1 to Sensitize Metastatic Colon and Breast Cancer Cells to Apoptosis Induction and Metastasis Suppression

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Reviewer: Daniele Lecis

Reviewer’s report:

In the manuscript “Ceramide Targets xIAP and cIAP1 to Sensitize Metastatic Colon and Breast Cancer Cells to Apoptosis Induction and Metastasis Suppression”, Paschall and colleagues show that the ceramide analog LCL85 sensitizes metastatic human cancer cells to Fas-mediated apoptosis. The authors demonstrate that LCL85 administration causes the increase of C16 ceramide levels, the proteasomal-dependent degradation of XIAP and cIAP1, the down-regulation of the anti-apoptotic protein Bcl-xL, thus resulting in augmented sensitivity to Fas ligand in vitro. Moreover, two syngeneic lung metastasis models, experimental and spontaneous, are used to demonstrate that LCL85 can suppress in vivo the formation of metastasis and the growth of the primary tumor in the fat pad. The authors propose the use of LCL85 to sensitize tumor cells to FasL expressed by cytotoxic T lymphocytes.

In my opinion, the work presented is interesting in the cancer field, experiments are clear and the characterization of LCL85 anti-metastatic activity is novel. Nevertheless, there are a few points that should be addressed:

Major Compulsory Revisions

1. The mechanisms of LCL85 activity have been investigated in vitro using a panel of human colon carcinoma cell lines (along with the breast carcinoma cell line MDA-MB-231), while the in vivo experiments are performed with the murine Colon26 and 4T1 cell lines. Does LCL85 sensitize also Colon26 and 4T1 cells to FasL and cause the degradation of XIAP and cIAP1 in vitro?

2. Does LCL85 trigger the down-regulation of XIAP and cIAP1 also in vivo in the cancer cell lines employed?

Minor Essential Revisions

1. In Figure 6, it is shown that all cell lines tested are sensitive to BV6 treatment. This is surprisingly because only few cancer cell lines are known to response to Smac mimetic treatment as single agent. Moreover, which is the vehicle used to dissolve the compound and the final concentration of the vehicle?

2. In Figure 1A, 1B, 2A, 2B, 4A, 6, 7A, error bars are missing especially in untreated controls. Furthermore, in Figure 2A, 2B and 7A, the same 100 % viability bar is used both for untreated cells and for LCL85-only (or BV6-only) treatment. From the other graphs, it is predictable that the administration of LCL85 and BV6 should affect, even if slightly, the viability of the cells, and
therefore it should be preferable to show the % viability relative to untreated and not 100 %.

Discretionary Revisions
1. In the first paragraph of the results session the authors divide the panel of cancer cell lines in primary (6) and metastatic (5) and state that 4 (SW480, HT29, HCT116 and LS174T) out of 6 primary cell lines are sensitive to FasL, while only one metastatic cell line (T84) respond to it. From Figure 2B the “clustering” of the two groups does not seem so obvious (e.g. primary LS411N, Colo 201 and Colo 205 seem to respond as HT29). Is the difference of sensitivity among the two groups noteworthy?
2. In Figure 8, the siRNA of cIAP1 and XIAP is tested by RT-PCR, but western blot would be a better way to show the efficacy of down-regulation of the targets.

Minor issues not for publication
1. At the end of the first result paragraph, authors refer to Figure 2A and 2B, but the figure with MDA-MB231 is Figure 3.
2. The Legend of Figure 5D is missing.
3. There are a few typographical errors throughout the manuscript: e.g. “subtoxic” with capital letter in results section of the abstract, space missing in the same paragraph “cIAP1protein” and before many references in the text, “1x10^4” written wrong in M&M, BV6 is called BV8 in the results paragraph “Ceramide analog and Smac mimetic additively…”, 4 full stops in a row in legend Figure 12

Level of interest:An article of importance in its field

Quality of written English:Acceptable

Statistical review:No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests