Author's response to reviews

Title: M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide

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M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide
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We would like to thank the reviewer n°2 for her/his helpful comments. We took into account all the comments in order to make the manuscript more clear, with a more precise interpretation of the results.

A point by point response to each of the comments is detailed here under.

Reviewer n°2

Figure 9 and 10 included enough evidences for apoptosis analysis. The inhibition effects of M2 in etoposide-mediated cancer cell death are convincible.

We would like to thank the reviewer for this comment.

However, the explanation of the M1 part is not satisfactory. From the western blot, M1 indeed increased etoposide-induced apoptosis in HepG2 cells but not in A549 cells. So please think about the conclusion for M1 macrophages.

M1 macrophages increased the etoposide-induced HepG2 cell apoptosis (Fig9) as well as the etoposide-induced A549 cell apoptosis as measured by caspase-3/7 activity (Fig10B) and propidium iodide-annexin V-labeling (Fig10C). However, no significant effect was observed on the cleaved caspase-3 and cleaved PARP-1 protein abundance in A549 cells (Fig10A), which is indeed no really consistent with the two other observations. However, for two western blot analyses out of the three independent experiments, this effect was observable (one of these analyses is illustrated in the Figure 10A). This is now clearly mentioned in the revised version of the manuscript.

Meanwhile, why M0 macrophages exhibited different effects in Fig9 and Fig10?
The authors should discuss it in the manuscript.

M0 macrophages had no effect on the etoposide-induced HepG2 cell apoptosis (Fig9) and no effect on the etoposide-induced A549 cell apoptosis as measured by caspase-3 and PARP-1 cleavage (Fig10A) and propidium iodide-annexin V-labeling (Fig10C). However, an increase was observed in the caspase-3/7 activity analysis in A549 cells (Fig10B), which is indeed no really consistent with the two other observations. This may be due to the activity of other caspases like caspase 7. This is now clearly mentioned in the revised version of the manuscript.

The marker label also needs to be adjusted in Fig 9A.
As suggested, the marker labels (molecular weight) have been adjusted in the revised version of the manuscript.

Please use consistent symbols for statistical significance, like “star”. Too many different symbols make people confused.

As suggested, the symbols for statistical significance have been simplified. However, we kept two different symbols for analyses performed on linear data and on log-transformed data. Accordingly, the description of the statistical analyses in the “Materials and Methods” section and in the legends of the figure have been changed.

Please adjust the location of the significance stars in Fig4A Fibronectin 24h.
As suggested, the location of the significance stars has been adjusted in the revised version of the manuscript.

Needs some language corrections before being published.
As suggested, the manuscript has been thoroughly checked for English language.