Author's response to reviews

Title: Histone deacetylase inhibitors strongly sensitise neuroblastoma cells to TRAIL-induced apoptosis by a caspases-dependent increase of the pro- to anti-apoptotic proteins ratio.

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Author's response to reviews: see over
Dear Editor,

Thank you for considering our work and for your prompt answer. You will find enclosed a revised version of the manuscript entitled “Histone deacetylase inhibitors strongly sensitise neuroblastoma cells to TRAIL-induced apoptosis by a caspases-dependent increase of the pro- to anti-apoptotic proteins ratio” by Annick Mühlethaler-Mottet, Marjorie Flahaut, Katia Balmas Bourloud, Katya Auderset, Roland Meier, Jean-Marc Joseph and Nicole Gross, to be reconsidered for publication in BMC Cancer.

The reviewers comments and suggestions were addressed in the revised version of the manuscript. In addition we wish to answer here the reviewer’s questions and explain our corrections point by point:

Reviewer: Thomas Griffith

Major compulsory revisions:

The major concern applies to the analyses of caspases activation in Figure 3B and Figure 4A. In these figures, we show a reduction of the pro-caspase expression levels (by western blot) induced by combined TRAIL and HDACIs treatments. We have interpreted this decrease of the pro-form as a pro-caspase cleavage. In order to confirm that this indeed correspond to caspases activation, we have performed caspases activity assays using colorimetric caspases substrates of caspases-3/7 for Figure 3C and colorimetric caspases substrates of caspases-8, -2, -3/7, and-9 for Figure 4B. We privileged such assays, which are equivalent to flow cytometric analyses of caspases activation proposed by the reviewer, since they allow the analysis of caspases cleavage by western blots and caspases activities by colorimetric assays in the same protein extract. In such conditions, we observed an increase of caspases activities (Figure 3C and Figure 4B) and a corresponding reduction of pro-caspases expression level in western blots (Figure 3B and Figure 4A). We are therefore confident that the decrease in the levels of pro-caspases observed in western blots results from caspases activation rather than from deregulation of the pro-caspase expression level, as mentioned in p12 lanes 13-15 of the Results section.
Finally, the reviewer suggested that decrease of pro-caspases could be caused by the release of proteins due to cell death. The western blots were performed with protein extracts from cells treated for a maximum of 16h. At this time point the percentage of cell death is less than 20% as shown in Figure 3B, whereas cell death occurs later (between 24 to 48 h). Therefore, the production of a significant release of proteins due to cell death would be unlikely at 16h.

Reviewer: Roberto R.Rosato

Minor essential revisions:

- All the legends corresponding to the different bars of graphs were included directly in Figure 1 (A, B and C), Figure 2A, Figure 3A, Figure 4B, and Figure 6B, as suggested. The corresponding legends were suppressed in the Figure legend section of the manuscript.
- For treatment with TRAIL, it is indicated in the Methods section that "cells were incubated with indicated amount of soluble recombinant TRAIL … and cross-linking mouse anti-Flag Ab M2 … with a constant ratio of 1/5 of TRAIL to M2 respectively". In addition, in all legends to Figure the dosage of TRAIL and M2 are indicated except for Figure 1A, C and Figure 6B. So, in the revised manuscript, the dosage of M2 was added in the legend to Figure 1C. Concerning Figure 1A and Figure 6B, only the dosages of TRAIL were indicated in the Figure to simplify these figures, but M2 dosages could be added if needed.
- In Figure 5C: Bcl-x was replaced by Bcl-xL.
- In Figure 6B: as suggested, only the results obtained with 100 nM of survivin siRNA are shown in the revised Figure 6B and the results with 25 nM of survivin siRNA are now mentioned as data not shown in p.15, lane 13 of the Results section.
- Figure 5b and 5c: As proposed, we have repeated the western blot for XIAP to improve the quality of the presentation for a better understanding of the results.

We hope that our explanations to the reviewers comments are clear and satisfactorily answer their questions, and that the modifications introduced in the manuscript have improved its scientific level to be suitable for publication in *BMC Cancer*.

Yours sincerely,

Annick Mühlethaler-Mottet