Author's response to reviews

Title: CD40L induces multidrug resistance to apoptosis in breast carcinoma and lymphoma cells through caspase independent and dependent pathways.

Authors:

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Author's response to reviews:

Editorial Office
BMC Cancer

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Dear Editor,
Thank you very much for your mail of December, 01st, 2005 regarding our manuscript entitled: "CD40L induces multidrug resistance to apoptosis in breast carcinoma and lymphoma cells through caspase independent and dependent pathways" by Nathalie Voorzanger-Rousselot et al (MS:108358707809814). We would like to thank the reviewers and the editors for their helpful comments and questions which we carefully examined. We have made several modifications suggested by the reviewers to improve the new version of this manuscript. Therefore, we submit a revised version of this manuscript as requested.

Reviewer 1:

Minor essential revisions

As it was suggested the term "caspase-3" activities has been changed in "caspase-3/7" throughout the article.

Reviewer 2

Major compulsory revisions

1 - It was reported that ceramides can act as second messengers for DNA damaging agents-induced apoptosis and they may act upstream or downstream of caspase. In the last part of the manuscript, we indeed propose that "the protective effect of CD40L does not involve a downregulation of intracellular ceramide production". This statement is based on the observation that in these experiments, caspase inhibitors inhibited C2 or C6 induced-apoptosis in breast carcinoma. If ceramides were second messenger of DOX-induced apoptosis in these models, caspase inhibitors should then inhibit DOX-induced apoptosis what is not the case. Although we cannot completely rule out a role for ceramide in DOX induced apoptosis, these results strongly suggest that ceramides is not a major mediator of DOX-induced apoptosis in this model. Since we did not investigated directly ceramide production in response to DOX and its modulation by CD40L, we agree that this statement remains a likely hypothesis.

Minor essential revisions

2 - We notified in the Results part that the anti-proliferative effect of C2 and C6 ceramides on the breast carcinoma and NHL cell lines tested was significantly reversed by co-culture with CD40L Lcells except for C2 in BT20 breast carcinoma cell line and Daudi and BL70 NHL cell lines.

3 - In the discussion part, we have suggested different mechanisms for CD40L protection against drug-induced apoptosis, in particular via Bcl-2-BclxL/Bax pathway but also via modulation of the cell cycle and the role of p53 family. Of note, the study of the downstream mechanism was further displayed in another publication.
Reviewer 3

Major compulsory revisions

1 - While caspase 3 activity was tested using three different techniques, in order to identify its genuine role (or not) in drug-induced apoptosis and drug resistance induction by CD40L, the investigation of downstream activity of C8 and C9 caspases was indeed performed using a single test. In order to not lose the main message of this work on caspase 3 activity in our model of NHL and breast carcinoma cell lines, the study of caspase 3 downstream mechanisms (C8, Bcl-2 family and p53) was further displayed in another publication.

2 - As it was suggested figures 1, 2 and 4 were modified to facilitate their reading and comprehension.

3 - Statistical analyses were added on the figures 1 and 4. Statistical differences (p<0.05) were indicated by the following symbol * and more explanation were provided in the Legends part.

4 - The variation of the protective effect of CD40L on the anti-proliferative effect of ceramides in cell lines was mentioned in the text of the article. The anti-proliferative effect of C2 and C6 ceramides on the breast carcinoma and NHL cell lines tested was significantly reversed by co-culture with CD40L Lcells except for C2 in BT20 breast carcinoma cell line and Daudi and BL70 NHL cell lines.

Minor essential revisions

5 - Abbreviations were suppressed in the abstract

6 - All abbreviations were defined in the text the first time used, in particular dihydrofolate reductase (DHFR) and methotrexate (MTX).

7 - We have reviewed the whole text in order to improve the quality of writing in English.

We hope that the additional comments presented in the new version as well as the modifications in the text will enable this manuscript to be acceptable for publication in BMC Cancer. We remain at your disposal for any further information regarding this work. Thanking you in advance for the attention you will pay to this work. Sincerely yours.

Jean-Yves Blay Nathalie Voorzanger-Rousselot