Author's response to reviews

Title: Compound K, a metabolite of ginseng saponin, induces apoptosis via caspase-8-dependent pathway in HL-60 human leukemia cells

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Author's response to reviews: see over
Dear Editor

We appreciate reviewers’ valuable comments and have revised our manuscript accordingly. Below, we address point by point how we respond to reviewers’ comments, and we hope that the revised form is now adequate for publication in your journal.

Reviewer’s report I

This study aimed to elucidate the molecular mechanism of compound K, a metabolized form of protopanaxadiol ginsenoside, induced cell death in leukemic cells. The authors chose the human promyelocytic leukemia HL-60 cell model to address this question and found that compound K mainly act via the caspase 8 controlled extrinsic pathway. Although several findings are already published this study shows in well performed experiments that a) compound K acts mainly via caspase 8 activation, b) capase 9 is activated by tBid/mitochondrial pathway and c) DISC formation is involved in caspase 8 activation.

Minor Essential Revisions:
1) Compound K induced apoptosis happens within hours and more remarkable at the highest concentration used in this study all cells become AnnexinV positive within 30 minutes and AnnexinV/PI positive within 4h. Using cycloheximide experiments the author hypothesized that de novo protein synthesis is required for compound K induced cell death. However, rapid apoptosis induction argues somehow against de novo protein synthesis (and immediate transcriptional response?). If cycloheximide inhibits activation of caspase 8, these cells should be protected from compound K induced cell death, what supports the hypothesis. The authors shall include data of apoptosis of cycloheximide-treated vs. non-treated cells exposed to compound K and shortly discuss the obtained data.
   -> In figure 6B, pretreatment of the cells with cycloheximide attenuated the compound K-induced DNA fragmentation, the hallmark of apoptosis. This data suggested that de novo synthesis might be required for compound K-induced cell death. Discussion regarding to this data has been described in the discussion part (pp 20-21 of the revised manuscript, highlighted with yellow color).

2) page 20, line 3: “….or by cleaving BH3,…..” . The author must clarify what is meant by ‘BH3’ (BH3-only protein Bid?)
   -> It has been clarified in the revised manuscript.

Discretionary Revisions
1) The authors showed ‘specific’ apoptosis in figure 1B rather than ‘total’ apoptosis, which include basal apoptotic levels of cell culture cells. In this figure the legend of the y-Axis shall be changed to ‘specific apoptosis (%)’.
   -> The legend of the y-Axis has been changed as the reviewer suggested in the
2) Apoptosis induced by compound K is mainly mediated by caspase 8 activation, as specific inhibition of caspase 8 protected the cells from apoptosis. The finding that the intrinsic pathway is involved via tBid and caspase 9 activation might also be explained by the hypothesis that tBid/caspase 9 activation is a consequence of active caspase 3, which is processed within 1h after treatment (figure 2A).

> This is a good point. The possibility that active caspase 3 is involved in the activation of tBid/caspase 9 has been briefly described in the discussion part (pp 18 of the revised manuscript, highlighted with yellow color).

**Reviewer’s report II**

Cho et al. aim to identify the specific mechanisms by which Compound K is purported to exhibit its anti-tumour activity. A series of detailed experiments (MTT assay, DNA fragmentation assay, PI & Annexin V double staining, mitochondrial membrane potential determination, protein extraction & w. blot, immunoprecipitation) were performed to elucidate the pathways and substrates responsible for apoptosis. This effect was identified as being caused by initiator- and executioner-caspases, induced via the mitochondria and primarily responsible for the apoptotic properties of Compound K in human leukaemic cells. The studies are performed and presented in a clear and methodical manner and the discussion that follows is sound in the explanation of the data that has been presented in Figures 1-6. Some minor (essential), primarily grammatical revision is required prior to publication:

1) All terms such as Bid, Fas, Bcl-2 etc etc.. should appear in ‘italics’ throughout the manuscript

> To the best of our knowledge, the name of gene (DNA), but not protein, should appear in ‘italics’. All the terms including Bid, Fas, Bcl-2 etc in our manuscript are for the protein levels. In addition, we failed to find any special guideline for the font style of DNA and protein levels in BMC cancer journal website. If it is our misknowledge, please, let us know so that we will be able to change the style of all the terms into ‘italics’ before the publication.

2) Change ‘activations’ to ‘activation’ throughout the manuscript

> It has been changed in the revised manuscript.

3) Change ‘compound K’ to ‘Compound K’ throughout the manuscript

> It has been changed in the revised manuscript.

4) Change ‘ul’ to ‘uL’ throughout the manuscript

> It has been changed in the revised manuscript.

Thank you very much for your advices.

Sincerely yours,
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