Author's response to reviews

Title: TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells

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Author's response to reviews: see over
Re: Ms. No. 1137693371025881 - TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells

Dear Prof. Dr. Shi-Yong Sun, dear Ms. Battad,

In response to your e-mail from December 3rd, 2013, we herewith resubmit the second revision of our above manuscript.

Following the suggestions of the handling editor, we have revised our manuscript once more and we sincerely hope that it is now suitable for publication in BMC Cancer. Please see our point-by-point response below for details.

I herewith affirm that all authors concur with the submission and that the material submitted for publication has not been previously reported and is not under consideration for publication elsewhere.

Please do not hesitate to contact me if there are any questions.

Yours sincerely,

Prof. Dr. Dieter Adam
Response to Editor’s Comments, Ms. No. 1137693371025881 - TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells

We thank the handling editor for his helpful suggestions.

A detailed point-by-point reply to his individual comments and a detailed description of the changes we made in the second revision of our manuscript follows below.

Comment #1: “TRAIL-induced programmed necrosis in principle occurs under the condition that the normal apoptotic pathway is inhibited. Therefore, it is helpful to add a discussion on under what conditions this strategy is potentially valid (e.g., caspase-8 mutation or inactivation, combination with a caspase inhibitor and so on) for treatment of cancer since this study focuses on TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells.”

Reply #1: We have expanded the “Discussion” section of our manuscript by the following passage: “Since this study focuses on TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells, we explicitly want to point out that TRAIL-induced programmed necrosis in principle occurs under the condition that the normal apoptotic pathway is inhibited. It has recently become clear that caspase-8 suppresses programmed necrosis under normal conditions and that it needs to be actively inhibited (e.g. by zVAD-fmk) for programmed necrosis to be executed. Notably, even the basal activity of non-stimulated caspase-8 is already sufficient for the suppression of programmed necrosis [20]. Therefore, the induction of programmed necrosis in apoptosis-resistant cell lines in the absence of caspase inhibitors would only be effective in tumors that carry a mutation that directly inactivates caspase-8. In all other cases (i.e. in cells that harbor apoptosis-inhibiting mutations affecting other proteins) the residual activity of caspase-8 would still be sufficient to suppress programmed necrosis. Most likely, this is the reason why the application of TRAIL alone has so far not been effective against apoptosis-resistant tumors in clinical trials. Therefore, we consider the inhibition of caspase-8 as an essential prerequisite for the successful elimination of tumor cells by TRAIL-induced programmed necrosis. In future treatment regimens this could be most conveniently achieved by combining TRAIL with a caspase inhibitor such as zVAD-fmk. With regard to its physiological and clinical relevance, zVAD-fmk has so far proven to be a non-toxic substance that has no adverse effects and which is well tolerated when administered for prolonged periods of time [3, 38, 39]. However, although TRAIL and zVAD-fmk by themselves have not shown toxicity in vivo [9, 40], it must be clarified whether their joint application (and additionally in combination with chemotherapeutic agents) is equally non-toxic in vivo.” and included the novel references #38 and #39 in our manuscript.
**Comment #2:** “TRAIL alone including combination with a chemotherapeutic agent will primarily induce apoptosis under normal conditions. We have to be clear that TRAIL/VAD/CHX regimen is not equal to TRAIL alone treatment here to avoid possible confusions as the second reviewer concerned.”

**Reply #2:** Following the handling editor’s suggestion, we have (wherever appropriate) changed all occurrences of “TRAIL-induced programmed necrosis” (or similar phrases, equally for TNF) to “TRAIL/zVAD/CHX-induced programmed necrosis” to additionally distinguish this kind of treatment regimen from treatment with TRAIL alone. To make the point raised by the handling editor even more clear in the manuscript, we have changed passages in the “Abstract” (Background) section to “Programmed necrosis is an alternative, molecularly distinct mode of programmed cell death that is elicited by TRAIL under conditions when the classical apoptosis machinery fails or is actively inhibited”, in the “Background” section to “As a potential alternative, we and others have previously demonstrated the ability of human and murine TRAIL receptors to induce programmed necrosis independently from their apoptotic capabilities when induction of apoptosis fails or is actively inhibited [7, 10]”, in the “Results” section to “This treatment is not only experimentally required to suppress apoptosis, but in addition potentiates programmed necrosis by inhibiting caspase-8, which acts as a negative regulator of programmed necrosis [20] and which otherwise would prevent the induction of programmed necrosis by TRAIL.”