Author’s response to reviews

Title: Simultaneous modulation of the intrinsic and extrinsic pathways by simvastatin in mediating prostate cancer cell apoptosis

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Author’s response to reviews: see over
Response to Reviewer’s comments:
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Title: Simultaneous modulation of the intrinsic and extrinsic pathways by simvastatin in mediating prostate cancer cell apoptosis

Version: 3 Date: 2 July 2012

Reviewer: Xu Dong Zhang

Reviewer's report:

The authors have addressed most of my concerns. However, it remains confusing that they could not detect BimEL in Figure 2A, while this Bim isoform is the most abundant form of Bim in cells. The authors should elaborate this in more detail. Another worry is Figure 5, in which caspase-9 blot is still not in publishable quality. This needs to be improved.

Response: Antibody that detects Bim (Cell signaling, #2819) identifies all the Bim isoforms. We identified the differences in BimL and BimS based on the molecular weight. We knew that BimEL is the major isoform in cells and since we did not see this protein expressed in prostate cancer cells, we initially reported this as changes in Bim as a whole and not as specific isoforms. This could be due to multiple reasons such as specificity of antibodies to certain isoforms, less expression of BimEL in prostate cancer cells, changes in their expression levels in hormone-insensitive cells compared to androgen-sensitive cells etc. Although the specific role of Bim isoforms in prostate cancer cells is less investigated, earlier studies indicates a 4th isoform specific to prostate cancer cells called Bim gamma (Jun-Wei Liu et al, Cancer Research, 2002). Our antibody did not detect this 34KDa isoform either. Since our study was not focused on Bim, we did not attempt detailed characterization of this molecule and include a detailed discussion based on this in our manuscript. A separate study on Bim isoforms in prostate cancer cells may be necessary to solve this mystery.

Caspase-9 blot in figure 5 was repeated 4 times and we received the same quality figures due to unknown reason. We have presented the best figure out of the 4 different experiments that clearly show the expression levels of cleaved caspase 9. We have now also confirmed this expression with changes in Caspase 9 activity.

Reviewer: Vladimir Ivanov

Reviewer's report:

The Authors have properly addressed the most of comments and substantially improved this manuscript. I still have two concerns.
1. One of the important observations of this work is up-regulation of FasL expression by simvastatin. The physiological consequence of this event, however, was not investigated. I still think that evaluation of the effects of anti-FasL antibody in the cell culture on simvastatin-induced apoptosis must be performed.

Response: We agree with the reviewer that up-regulation of FasL must be accompanied with its functional effects on apoptosis. Our data showing increased expression of cleaved caspase-8 is also supportive of this conclusion. However, from our experience with the experiments involving the effects of statin on the intrinsic pathway, overexpression with Bcl2 and/or DN-Caspase9 did not rescue the effects of simvastatin on prostate cancer cells. This suggests that re-constituting with either of the intrinsic or extrinsic pathway alone may not be enough for a rescue. Even if the experiment with anti-Fas ligand antibody fails to rescue, this still does not rule out that FasL is not involved in the process since statin can also generate cleaved caspase 3 utilizing the intrinsic pathway. For this reason, we did not perform this experiment during the previous round of revision.

2. The last concern is regarding Fig.9 that summarizes the main results of this study. There are several points of this picture, which do not reflect precisely apoptotic mechanisms: TNF inside the cell, the intrinsic pathway outside of the cell, JNK?? – What does it mean?

Response: We completely agree with the reviewer on this comment and apologize for the errors. We have attached a new figure 9 with this submission.

Authors hope that we have sufficiently answered reviewer's critiques and that now the manuscript is ready for publication.

Thanks

PR Somanath