Reviewer's report

Title: Synergetic downregulation of 67kDa laminin receptor by the green tea (Camellia sinensis) secondary plant compound epigallocatechin gallate: a new gateway in metastasis prevention?

Version: 3 Date: 24 August 2012

Reviewer: Bastian Hoesel

Reviewer's report:

The authors pose the question whether a major green tea secondary plant compound EGCG has inhibitory aspects on the expression of the metastasis-associated 67kDa laminin receptor.

The major finding presented is a synergistic effect on 67LR expression in 67LR siRNA virus EGCG treated JPEC-12 cells compared to EGCG treated control virus treated JPEC-12 cells.

First the authors present a graph were the respective 67LR expression data has been normalized to a non-targeting virus control and show that there is an enhanced down-regulation of 67LR expression in EGCG treated 67LR siRNA virus treated JPEC-12 cells compared to control cells.

Furthermore the effect appears to be specific for 67LR since a “control-vector” does not show this effect.

Then the authors use the same data as presented in Figure 1a and normalize it to a “media control”. By that the authors calculate the net effect of the synergistic 67LR down-regulation.

Furthermore the authors use principal component analysis to confirm their basic findings by an alternative method.

Finally the authors show that the synergistic effect appears to be EGCG dose dependent.

Major Compulsory Revisions:

1. The term media control is misleading since it indicates an EGCG control without virus treatment. It would be more convenient (and also easier for the reader) to indicate directly to which sample the respective treatments have been normalized to (for example in the Figure legend).

Figure 1a: MC/TV was normalized to MC/NV. ET/TV was normalized to ET/NV.
Figure 1b: ET/TV was normalized to MC/TV. ET/NV was normalized to MC/NV.
2. For further approval the authors show the measured effect by applying a descending concentration series of EGCG. Why are the given results distributed in two plots?

3. The passage around line 250 describes the anti-metastatic potential of green tea to be most effective in the gastrointestinal tract. Is it not possible to postulate this thesis on the base of this study.

4. Why does treatment with EGCG “alone” show absolutely no effect and why is the effect only present after the lentiviral knockdown? This should also be included in the discussion.

5. Why is/are no target gene(s) of the 67LR pathway included in the study? This would be good to confirm the effect and also to shed light on the underlying mechanism.

Minor Essential Revisions:

1. The title might be improved by using the more common synergistic instead of synergetic.

2. It is misleading to postulate a new gateway in metastasis prevention since the enhanced inhibitory aspects of green tea is only found after initial artificial down-regulation of 67LR which might be difficult to achieve in patients.

3. In Figure 1a the y-ordinate is labelled as “knockdown”. Would it not be more precise to use the term knock-down efficiency?

4. Figure 2 could be improved by indicating the groups in the Figure legend and by using independent Figure legends for each graph.

The effect presented in the study is certainly interesting and worth publishing since to my knowledge the concept of a specifically enhanced down-regulation of a gene by a plant compound, which is not due to a general enhancement of the RNAi machinery has not been published before.

Nevertheless I think the biological background the authors put their data in does not really stand on solid ground and is not sufficiently supported by the data. It would thus be necessary to attenuate their findings in regards to the prevention of metastasis spread.

As a consequence the manuscript could be much improved by adding data which sheds light on the underlying mechanism and also better integrates the data in the biological context.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests