Reviewer’s report

Title: Snake venom toxin from Vipera lebetina turanica induces apoptosis in colon cancer cells via upregulation of ROS- and JNK-mediated death receptor expression

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Reviewer: Steven de Jong

Reviewer’s report:

Park et al. have written an interesting article on snake venom toxin as a drug activating DR4 and DR5-induced apoptosis. The induction of apoptosis by snake venom toxin is well proven and mechanistical insights are provided. However, the most critical experiment, which should proof the direct relation between venom toxin and activation of DR4/DR5-mediated apoptosis is, i.e. the DR4 and DR5 siRNA experiments are performed in the presence of TRAIL. Therefore I’m not convinced that the snake venom toxin induces apoptosis via activation of DR4 and DR5 apoptosis signaling.

Major compulsory revisions
1. The background is too long and has to be shorthened.
2. In figure 2 the DR4 and DR5 cell surface expression and the venom toxin-induced change in DR4/DR5 expression at the cell surface has to be shown.
3. Quality of the caspase 8 blot (figure 3) needs to be improved. Capsase 3 blots are not very clear, isn’t it possible to use PARP cleavage or another marker downstream of caspase 3 to indicate apoptosis induction?
4. In figure 4 the authors have used DR4/DR5 siRNA in combination with TRAIL. How much TRAIL was used? What happens in the absence of TRAIL with the level of apoptosis induced by venom toxin in this experimental setting. In addition, did the authors test a TRAIL blocking antibody to define whether the apoptosis is due to paracrine/autocrine induction of TRAIL-DR4/DR5 complex formation? Did the authors measure cell surface expression of DR4/DR5 at the cell surface after siRNA treatment?
5. P-JNK blots are not very indicative (figure 5C and 6B). The induction seems to be less than what was shown in figure 5A.
6. References have to be checked carefully, many errors in the names or not all names are included in the reference.

Minor essential revisions:
LY303511, a phosphoinositide 3-kinase (PI3K) inhibitor. This is an inactive drug which cannot inhibit PI3K.
Some typos
- 8Snake venom toxin (in title)

Many errors in sentences, for instance
- Recently, many research suggested that the down-regulation or mutation of death receptors has been proposed as a mechanism by which cancer cells avoid destruction by the immune system.
- Therefore, it is very important that finding the agents that increases the death receptors of cancer cells for decrease of resistance changes of CCD18 Co normal colon cell growth

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

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