Reviewer's report

Title: Rho-kinase inhibitor HA-1077 suppresses the proliferation/migration and induces apoptosis of urothelial cancer cells

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Reviewer: Martina Schmidt

Reviewer's report:

Yoshida and colleagues described in their current study the impact of the Rho-kinase inhibitor HA-1077 on the proliferation/migration of the urothelial cancer cell lines 5637 and UM-UC-3. The authors provided evidence that HA-1077 inhibits the elevation of ROCK-1 and ROCK-II expression, leaving RhoA activity unchanged. As an additional control GGOH was used to modify the posttranslational modification of RhoA. In addition, effects of HA-1077 on proliferation/migration of the cancer cell lines seem to discharge on the level of apoptosis. Overall the studies being performed are interesting, and add novel insight into the effect of the Rho-kinase inhibitor HA-1077. Evidence is provided that HA-1077 might be a useful tool in cancer treatment. The effect of HA-1077 on the expression level of ROCK is rather interesting. Although any underlying mechanism is highlighted.

The manuscript needs improvement in some points before being considered for publication:

Major points:

1. Figure 1, throughout the manuscript. It is not clear whether the effect of HA-1077 alone has been studied on the level of Rho activity as well as expression of ROCK-I/ROCK-II. Most likely in Figure 1, HA-1077 has been studied alone and in combination with LPA and GGOH. In Figures 2 and 3 it is not clear whether HA-1077 has been studied alone or in combination with LPA. Please clarify.

   If HA-1077 has been studied alone on the level of Rho activity one would not expect an effect on Rho activity on its alone but only in combination with LPA as GTP loading of RhoA has to occur. In Figures 7 and 8, HA-1077 seem to block GTP-loading by LPA. Although the authors claim that HA-1077 does not modulate RhoA activity? Please clarify.

2. Please clarify the purpose of HeLa cells being used as control. What is the negative control.

3. Please clarify the purpose the Figure 2. In the Figure legend it is stated that HA-1077 inhibits proliferation. Illustrated here is a cell layer?

4. Please comment on the use of the urothelial cancer cell lines 5637 and UM-UC-3. Rational?

5. It would be easier for the readership if the Result section would be a little bit
more detailed.
6. Please include into the Discussion a section about the potential mechanisms leading to the reduction of ROCK expression by HA-1077.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

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