Reviewer’s report

Title: Incarvine C suppresses proliferation and vasculogenic mimicry of hepatocellular carcinoma cells via targeting ROCK inhibition

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Reviewer: Kriengsak KL Lirdprapamongkol

Reviewer’s report:

- Major Compulsory Revisions

1. The authors provide misinterpretation for the anti-VM effect of IVC.

   In the author’s previous report (ref. 17; PloS one 2014, 9(9):e107661), the authors clearly showed that ROCK inhibition by Y27632 (50 microM) could inhibit only cell migration, invasion, and VM formation of MHCC97H cells without apoptosis induction (24 h) or inhibiting cell proliferation (48 h). This indicates that ROCK does not essential for survival and proliferation of MHCC97H cells.

   In this manuscript, the suppression of cell proliferation and apoptosis induction by IVC were clearly demonstrated at a concentration range of 15-60 microM. As shown in Figure 3, IVC at 15-30 microM significantly induced apoptosis after 24 h treatment. IVC also inhibited cell proliferation (48 h) as shown by IC50 value of 35 microM in Figure 1B.

   In this manuscript, the inhibitory effects of IVC (at 15 and 30 microM) on MHCC97H cell motility, invasion, and VM formation are parallel with cytotoxicity of IVC. These results of IVC are difference from the results of the Y27632 positive control ROCK inhibitor which showed in the author’s previous report (ref. 17). Taken together, these results indicated that the inhibitory effects of IVC on cell migration, invasion, and VM formation are associated with cytotoxicity of the compound. Any cytotoxic compound such as doxorubicin can also inhibits migration or VM at its cytotoxic concentrations, due to the cellular damage.

   Thus it is not clear that the inhibitory effects of IVC are due to ROCK inhibition or cytotoxicity of the compound. If the authors can provide the results demonstrate that ROCK inhibition leads to suppression of cell proliferation and apoptosis in the MHCC97H cells, the conclusion that IVC inhibits VM through ROCK pathway will be accepted although it contrast to the author’s previous report.

2. Although IVC could inhibit ROCK activity but it may affect other cellular targets at the same time. The cell cycle arrest and apoptosis induction of IVC may caused by effect of compound on the other targets. This point is an interesting to explore further.

- Minor Essential Revisions

1. Abstract/line9 : 7-AADassay (typographical error)

2. Figure Legends: The authors should inform how long the time that cells were
incubated with IVC before harvesting for analysis in Figure 4A, Figure 6C, and Figure 6E.

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

- Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?
  
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  No

- Do you have any other financial competing interests?
  
  No

- Do you have any non-financial competing interests in relation to this paper?
  
  No

I declare that I have no competing interests.