Title: Neuropilin-2 Induced by Transforming Growth Factor-beta Augments Migration of Hepatocellular Carcinoma Cells

Reviewer: Sanjay Katiyar

In the manuscript entitled "Neuropilin-2 Induced by Transforming Growth Factor-beta Augments Migration of Hepatocellular Carcinoma Cells" Whittmann et al, have attempted to investigate the role of NRP2 (that is upregulated through TGF-/Smad signaling) in HCC tumor cell migration and invasion.

From the experiments it was concluded that NRP2 expression was upregulated by the canonical TGF-/Smad signaling but it did not impact on TGF-# signaling in HCC cells. Reducing the NRP2 expression using siRNA based knock-down or inhibition of TGF-# signaling via LY2109761, a Type 1 & 2 TGFb receptor inhibitor resulted in diminished cell migration. This phenomenon appeared to work independently of each other. It was therefore concluded that NRP2 (possibly) does not collaborate with TGF-# signaling in cell movement.

The investigators had also tried to extrapolate the results obtained from in vitro experiment based observations to explain the enhanced NRP2 expression that was observed in the HCC tumor specimens and correlated that to higher tumor grade and decreased differentiation.

Although the experiments appear to be thoughtfully planned it is difficult to simply extrapolate the results from few in vitro experiments into the observations that were made in tumor specimens. A direct link between the two was not investigated through these experiments. The experiments described in the manuscript therefore should use the immunohistochemical NRP2 expression data from tumor specimens as supportive data that cannot be used in conjunction with in vitro data.

Also for any cell migration based in vitro investigations it is always prudent to use several parallel assays to prove the same hypothesis, which is missing in this manuscript. The authors did not provide a detailed procedure for conducting the wound healing assays, such as how the wound measurements were done and at how many points within the wound were measured to get an average data. The manuscript also lacks the detailed procedure for RQ-PCR, control gene expression that used to normalize the RQ-PCR data and the type of comparison that done among epithelial and mesenchymal making it difficult to understand and make sense out of Figure 1 D.
Major Issues: Also it was not clear from the manuscript text whether the experiments were performed once in triplicates or the data presented in the manuscript is from three independent experiments with at least 3 replicates in each experiment.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

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