Reviewer’s report

Title: Plexin-B1 silencing inhibits ovarian cancer migration and invasion

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Reviewer: Luca Tamagnone

Reviewer’s report:

This manuscript by Ye and coworkers addresses the functional role of Plexin-B1 (the receptor of Semaphorin 4D) in ovarian cancer cells. It is reported that Plexin-B1 is overexpressed in ovarian cancers with respect to normal ovary. Moreover, Plexin-B1 silencing experiments (by siRNA transfection) demonstrate that this receptor is required to mediate the migration and invasiveness of immortalized ovarian cancer cells.

The function of Plexin-B1 in cancer cells appears to be controversial and these data contribute to the discussion bringing evidence to the hypothesis that this receptor may act as a promoter of tumor progression. On the other hand, the present work should be developed and improved with further experiments to provide compelling evidence in support of the authors’ claims.

Major Compulsory Revisions:

1) In certain experimental models, it has been shown that PlexinB1 stimulation by Sema4D leads to increased cell migration and invasive growth, while in other cellular contexts the opposite seems to be true (e.g. Giordano et al., 2002; Barberis et al., 2004; Basile et al., 2005; Swiercz et al., 2008). To further elucidate this important issue, the authors should test the functional activity of Sema4D in multiple lines of ovarian carcinoma cells, relative to the expression levels of PlexinB1 receptor, and also upon receptor knock-down.

2) In order to confirm the role of PlexinB1 in cancer cell invasiveness, the authors should establish high expression of this receptor in ovarian cancer cells carrying low endogenous levels and test the functional outcome.

3) In order to elucidate the loss of function consequent to Plexin-B1 knock-down, cell viability/proliferation should be evaluated before cell migration/invasion.

4) It is not sufficient to show the effect of only one PlexinB1 siRNA transfected in ovarian cancer cells. Data should be shown concerning at least another siRNA (or shRNA) sequence, to establish specificity and rule out off-target effects.

5) Cytoskeletal changes observed upon sustained Plexin-B1 knock-down (Fig. 4) are explained here by implying that this signalling cascade normally elicits Rho activation in ovarian cancer cells. However, several other potential explanations can be put forward. Moreover, Rho regulation downstream to Plexin-B1 is a transient event and a rather controversial issue (e.g. see Barberis et al., 2005 and Swiercz et al., 2008). Thus, these data could be relevant if accompanied by a biochemical analysis of Rho activation in these cells (either upon Sema4D
stimulation, or PlexinB1 knock-down).

6) The last paragraph at the end of page 13 contains a wrong statement, since there is no evidence that Rho GTPases interact with the C-terminus of Plexin-B1 (only a PDZ-Rho-GEF).

Minor Essential Revisions:

7) English should be revised since the text contains a number of mistakes. In addition, some of the references appear to be in the wrong format.

**Level of interest:** An article of importance in its field

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

**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'