Reviewer's report

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

Version: 1 Date: 28 February 2010

Reviewer: Magali Williamson

Reviewer's report:

The authors of this manuscript have shown higher levels of plexinB1 protein expression in ovarian cancer tissue in comparison to normal ovarian tissue. The association between high levels of plexinB1 protein and ovarian carcinoma is statistically significant. PlexinB1 is also expressed in ¾ ovarian cancer cell lines. Knock down of plexinB1 by siRNA in one ovarian cancer cell line resulted in a reduction in Akt phosphorylation and a reduction in motility, invasive capacity and stress fibre formation.

This paper is interesting as it suggests that plexinB1 acts as an oncogene in ovarian cancer, as it may do in breast cancer and prostate cancer, in contrast to melanoma and renal carcinoma where it appears to act as a tumour suppressor gene. The methods used are appropriate and well described and the data is sound.

Major Compulsory Revisions

• Expression of plexinB1 in ovarian cancer has been reported previously by Valente et al. (Cellular Oncology, 31, 6; 2009) in a smaller series. PlexinB1 expression was found 9/15 ovarian serous carcinomas and 6/15 ovarian samples expressed both met and plexinB1 which was predictive for an unfavourable outcome. This should be discussed, and the statement ‘this is the first report examining plexinB1 in ovarian carcinomas’ (page 4) changed.

• The effect of knocking down plexinB1 is only shown for one ovarian cancer cell line. Ideally, to show whether or not this is a general phenomenon for ovarian cancer cell lines, the same experiments should also be done on other ovarian cell lines. It may be that the opposite effect is found, as in certain breast cancer cell lines (Swiercz et al 2008).

A reduction in Akt phosphorylation is seen upon knockdown of plexinB1 and correlates with the remaining plexinB1 expression levels, suggesting that activated plexinB1 contributes to Akt phosphorylation in this cell line. Is the activation of plexinB1 in these experiments the result of: 1. sema4D expression in SKOV3 cells, acting via an autocrine/paracrine mechanism? and/or 2. does activation of plexinB1 result from overexpression and consequent clustering of the receptor? Ideally phosphorylation of plexinB1 in response to sema4D should be shown in this cell line – this may not be possible however if Akt is constitutively activated. These points should be discussed.
Data should be shown for the effect of knockdown of plexinB1 with at least one of the other siRNAs used on migration and invasion.

Minor Essential Revisions

- The authors state that the protein is translocated from the cell membrane to the cytoplasm in carcinoma cells. The term translocation implies that the protein is actively transported from the membrane to the cytoplasm. No evidence has been shown for this, therefore it would be more accurate to simply state that the protein is expressed in the membrane and cytoplasm in carcinoma cells.

- Which santa cruz plexinB1 antibody was used?

- There are a few errors in the references:

  - Reference 31 should be referred to as Swiercz et al. rather than Jakub et al. In addition, the wrong reference is cited: Swiercz et al 2008 (concerning plexinB1 signalling via ErbB2 and Met) is reference 31 and should be cited instead of reference 44 in the discussion. Reference 44 is Korosteylev et al, (not Jakub/Swiercz et al.) (page 13)

  - The reference cited at the beginning of the discussion should be a review of plexin action, rather than reference 33.

  - In the discussion (To date, it has been proved that..................) As far as I am aware, PlexinB1 has not so far been shown to bind cdc42 or directly with RhoA. Also, the reference cited for this (2) is wrong.

Spelling – phosphorylation page 11

Grammar – some English grammar needs changing, eg. ‘plexinB1 has been reported to implicate in the process…. (page 11)

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

**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'