Reviewer's report

Title: The role of VEGF-A165b in trophoblast survival - implications for pre-eclampsia pathophysiology

Version: 2 Date: 17 January 2014

Reviewer: Carl Hubel

Reviewer's report:

Bates and co-workers have previously described a splice variant of VEGF-A165, namely VEGF-A165b that inhibits VEGFR-2 activation. This variant has anti-angiogenic properties in human cancers and in wound healing. The present report suggests a significant effect of VEGF-A165b as a survival factor for trophoblast cells under low oxygen conditions thought to occur during first trimester of pregnancy. The study entered on cell viability and cytoxicity, and lysate levels of VEGF-A165b by ELISA, under <2% and 20% oxygen culture conditions. A major tool used to distinguish isoform effects was the anti-VEGF-A165b antibody previously shown to recognize 165b and not 165.

While interesting and potentially important there are some suggestions and necessary clarifications.

Major:

1. The lower oxygen condition should be more precisely defined (can a range such as 1-2% be specified. This is important because anoxia could have dramatically different effects. Please also related the lower oxygen condition more clearly to the reported < 20 mm Hg state of first trimester placenta. Please also consider examining whether 165b concentrations in cell lysates differs between 1-2%O2 and an intermediate oxygen condition such as 8% O2 that is not hyperoxic (20% O2 is hyperoxic for these cells).

Minor:

1. Methods: There should be a section describing the ELISA. Presumably this was R&D DY3045. Previous studies suggest limited cross-reactivity to proangiogenic VEGF isoforms. Nevertheless the authors should provide some validation data for their cell lysates (e.g., intra- assay variability, dilutional parallelism, spike recovery).

2. The cellular levels reported (~2 ng/mL) differ greatly from the doses of VEGF-A165b used experimentally (40 ng/ml). Please justify the concentrations of exogenous 165b and use either nM or ng/ml in Methods and Results).

3. Given the relatively minor increases in lysate 165b after <2% compared to 20% O2, it remains difficult to understand why the anti-165b antibody had no cytotoxic effects under high oxygen conditions. Please clarify.

4. The Proliferation Assay is really a viability assay. How was it determined that
the cells in the media after 48 h were dead cells—or was this an assumption? How were live vs. dead enumerated—by hemocytometer?

5. The added value of the viability assay over the cytotoxicity assay is not clear.

6. Discussion, line 230. None of the data provide a reason why VEGF-A165b concentrations are low in patients that later develop preeclampsia. Please reword this.

7. Evidence for specificity of the anti-VEGF-A165b antibody was independently strengthened by Manetti M et al, Circ Res 2011 and should be discussed.

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