Reviewer's report

Title: Loss of Endocan tumorigenic properties after alternative splicing of exon 2

Version: 1 Date: 25 August 2007

Reviewer: William Aird

Reviewer's report:

The authors show that there are two isoforms of endocan in HUVEC, primate lungs and human lung tumors, that expression of the shorter form (endocan-2) in 293 Flip-in cells and HEK 293 did not result in increased tumor formation (vs. full length endocan), and that endocan-2 is associated with less glycanation. The study, while potentially interesting and important, raises a few concerns.

MAJOR

1. The authors should consider testing the effect of endocan-2 on tumor growth using a non-HEK cell line (e.g., HT29) as they did in their original report.

2. The authors should demonstrate whether endocan-2 increases tumor cell proliferation in vitro (as predicted by lack of effect of MEP08 on thymidine uptake in their original report).

3. In stable and transient transfections, endocan-2 resulted in less secretion of endocan. Lower secretion may have had a profound effect on tumor growth. Before concluding that “deletion of exon 2-derived sequence alters protein expression”, the authors should show comparable levels of transduction/transfection and endocan mRNA in the endocan and endocan-2 expressing cells. If the Flip-in cells control for copy number/integration site, they should state so (I could not find any description of this cell line on the Invitrogen website).

MINOR

4. The absence of detectable endocan in a single organ (lung) of mouse or rat using a semi-quantitative technique (RT-PCR) does not mean that the alternatively spliced form is primate-specific.

5. The authors should state whether/how the expression of the two endocan isoforms differs in human lung cancer vs. normal primate lung and primary endothelial cells.

6. The term “part-time” proteoglycan should be in quotation marks on page 4, as it is later in the Discussion.

7. There are many spelling/grammatical errors. For example, Rhesus Monkey should read Rhesus monkey (p 5); “translated in HUVEC in a 14 kDa” should read “translated in HUVEC into [or as] a 14 kDa…” (p 8); primate specific should
be primate-specific (p 8); endocqn (p 9); northern should be Northern (p 12)

**What next?:** Accept after minor essential revisions

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

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

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

**Declaration of competing interests:**

We have applied for a patent relating to the role of endocan as a tumor endothelial cell-specific marker in cancer