Reviewer's report

Title: Glioblastoma-derived Leptin Induces Angiogenesis and Growth of Endothelial Cells: Comparison with VEGF Effects.

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Reviewer: Daniel Stieber

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The manuscripts addresses the possible role of leptin, an adipocyte derived hormone involved in the regulation of food intake, in glioblastoma angiogenesis. It has previously been shown that leptin is an angiogenic factor in vitro and in vivo. Here the authors report leptin expression at the mRNA level in two glioblastoma cell lines, and protein expression in one of the two cell lines. Using the HUVEC tube forming assay, an in vitro surrogate assay for blood vessel formation, the authors show that conditioned medium of the glioblastoma cell lines induces tube formation and this can be blocked by a presumed antagonist to the leptin receptor. The authors propose that glioblastoma derived leptin plays a role in glioma angiogenesis.

- Major Compulsory Revisions

1) The main novel conclusion that the authors draw from their results is that leptin is a major angiogenic factor secreted from glioblastoma cells. Although this is a potentially interesting and important finding, the data presented are not sufficient to support this claim. Although it is clear from many studies that glioblastoma secrete several angiogenic factors (among which VEGF as the most notable one), their conclusion is entirely based on a novel inhibitor of the leptin receptor, for which the specificity has not been demonstrated. Thus in order to convincingly show that the angiogenic factor in the conditioned medium acts via the leptin receptor, a direct knockdown of the receptor by e.g. RNA interference in endothelial cells is mandatory. Alternatively a knockdown of the ligand in the tumor cells could be foreseen.

This is particularly important since it is not clear (and not discussed) why the conditioned medium from both cell lines has a similar angiogenic activity, while leptin can only be detected in one of them.

In this context it would also be interesting to report on the effect of the antagonist on the LN229 cells, which do not show detectable leptin levels.

2) It is well known that cell lines do not faithfully represent the tumor they are derived from. Since all experiments are based on cell lines, rather than primary tumor tissue, this should be clearly stated throughout the text. F. ex. ‘glioblastoma’ should be replaced by ‘glioblastoma cell lines’ throughout and most importantly in the title. Replace ‘angiogenesis’ by ‘tube formation’
throughout.

3) Statistical analysis and significance should be added for all experiments (e.g. Tables 1&2; qPCR experiments), and the statistical tests applied should be reported.

-Minor Essential Revisions

1) Figure 3C, include molecular weight indication on the Western blot.

2) The quantitative PCR graph (or the text) should give an indication of the levels of mRNA detected, meaning how does the level of leptin compare to that of VEGF. This could be done e.g. by indicating the threshold values of both transcripts.

3) The authors should address the discrepancy of the effect of CM from the two different cell lines in greater detail. Moreover there appears to be no clear correlation between the detectable levels of leptin and VEGF in the CM of the two cell lines and their tube forming potential on HUVEC cells.

4) The abstract states that VEGF was used at a concentration of 5µg/ml, which is an extremely high concentration for this highly potent agent. Later on in the text it is stated that it was used at 50ng/ml. Please clarify.

5) It would be useful to represent all tube formation assays in the same manner (Fig.1A indicates fold changes, while the tables 1&2 indicate % values).

6) In Fig. 2B the values appear highly variable and therefore rather unreliable (5 +/- 307pg/ml). Is this correct?

7) Fig. 3C: why were the cells in this experiment pretreated with the inhibitors, while for the functional assays they were not?

8) Reference 34 and 37 are the same.

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

Quality of written English: Acceptable

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