Reviewer's report

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

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Reviewer: Kai Ove O Skaftnesmo

Reviewer's report:

The article “Glioblastoma-derived Leptin Induces Angiogenesis and Growth of Endothelial Cells: Comparison with VEGF Effects” by Rita Ferla and colleagues is well written and examines important aspects of glioblastoma (GBM) biology. The prognosis for patients carrying GBM is still grim despite current advances in cancer treatment regimens. Therefore basic research on the biology of GBMs is important and may unravel molecular mechanisms that might be targeted in new therapeutic regimens. In this regard, the authors examines in their report GBM derived leptin and its effect on tumor angiogenesis. They first demonstrate that leptin is produced by GBM tumor cells in culture, secondly they show that this tumor derived leptin is able to induce angiogenesis in an in vitro Huvec tubule assay and that the effects is comparable to that of VEGF, the prototype angiogenic molecule. Finally they show that Aca1 an antagonist of the leptin receptor can block tumor induced angiogenesis in the in vitro HUVEC assay and that the effect can be enhanced by a coadministration of the VEGFR inhibitor SU1498. The methods used depend on in vitro model systems of angiogenesis and are adequate for addressing the questions raised by the authors.

- Major Compulsory Revisions:

The article is well written, interesting and addresses an important topic as it is related to the design of new therapeutic strategies targeting angiogenesis in GBMs. However a main weakness of the paper is that most of the findings can only be considered as small advances as the major findings in the paper have already been described previously. For example it has been demonstrated, although in other cancers than GBM, that tumor cells can make leptin and that leptin have a role as an angiogenic stimulator acting through the leptin receptor. It is also suggested in a previous article that leptin can potentiate VEGF's effect on angiogenesis.

The recommendation for publication is therefore that the authors extend their analysis so to make it more original.

The in vitro results reported in the article are encouraging and suggests that a dual administration of VEGFR antagonist and Leptin antagonist would be the most effective way to block tumor induced angiogenesis relative to single agent administration. As there exists limited reports examining how leptin inhibition would affect tumor progression in in vivo models, one suggestion to extend the
analysis would be by performing in vivo experiments were the authors examine the effect of Aca1 and SU1498 on intracranial brain tumor xenografts.

Alternatively or in addition the manuscript can be improved by additional in vitro experiments.

One weakness is the number of glioblastoma cell lines analysed for VEGF and leptin expression. As the authors only analysed two cell lines and only found one with robust leptin expression it will be valuable to know how the expression is in additional cell lines. This could be readily done by qpcr analysis, or public available expression databases could be mined in order to investigate this. Additionally it would be interesting to know how leptin production is regulated in cancer cells. As the author suggests this could be dye to physiological mechanisms like tumor hypoxia? An analysis of leptin levels in GBM cells lines before and after exposure to hypoxic conditions would clarify this. Utilising ELISA, the authors failed to detect Leptin and VEGF in one cell line LN229 and discusses the possibility of formation of complexes that cannot be detected by ELISA. A western blot analysis might clarify this question. It should also be considered that there is a possibility that the cell line perhaps only produces detectable amounts of VEGF and Leptin under hypoxic conditions. Another possibility is that this perhaps is dependent on cell density. The authors could therefore determine levels of Leptin and VEGF secreted from multicellular tumor aggregates (tumor spheroids) as this model better mimics in vivo tumors.

- Minor Essential Revisions

In the abstract under the background section it is stated that “Until present, the potential role of intratumoral leptin in GBM malignant spreading has not been addressed.” It is recommended to exchange “malignant spreading” for another phrase as malignant spreading is not the topic of the article. The phrase “malignant spreading” is also used in paragraph 4 in the background section and should also be substituted.

In the results under the section “Leptin and VEGF stimulate angiogenesis, growth and signalling in HUVEC. Inhibitors of ObR and VEGFR block these effects” it is stated in paragraph two that “However, no great influence on cell growth was detected in Huvec treated with Aca1 alone”. This statement is surprising given that the authors states in the sentence above that “...the antagonist at the highest concentration (50nM) produced cytotoxic effects, significantly more pronounced in the absence of leptin”.

In the next section “Effects of ObR and VEGFR inhibitors on CM-induced angiogenesis and growth of HUVEC”, there is an incomplete sentence in paragraph 3 reading “SU1498 at 5µM reduced LN18 CM-mediated growth of HUVEC by 20%, while no significant effects was observed with SU1498 1µM and higher concentrations of the antagonists were slightly cytotoxic”.

In the method section under “In vitro angiogenesis assay” a better description of the scoring of ES representing tube-like formation capability is needed. Alternatively a citation is needed.
In Figure 2A, the units of the Y-axis needs to be specified. I assume this is in %.

In Figure 3A, the concentration of the Aca1 and SU1498 should be specified either on the figure or in the figure legends. In Figure 3B abbreviations in the figure should be stated in the figure legends.

In Figure 4A, the concentration of the inhibitors should be mentioned, either in the figure or in the figure legends.

- Discretionary Revisions

Further, as to rule out any unspecific effects of the Aca-1 inhibitor it would be valuable to show the same effects by a complementing method as for example siRNA depletion of the leptin receptor in the HUVEC cells.

Declaration of competing interests:

The referee declare that he have no competing interests.