Reviewer's report

Title: Lipid Metabolism Enzyme ACSVL3 Supports Glioblastoma Stem Cell Maintenance and Tumorigenicity

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Reviewer: Antonella Rosi

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Lipid Metabolism Enzyme ACSVL3 Supports Glioblastoma Stem Cell Maintenance and Tumorigenicity
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General Comments

There is growing evidence that cancer stem cells play an important role in tumour initiation and recurrence. Improved knowledge of their response to metabolic modulation may provide new and more specific therapeutic strategies. Cancer stem cells are able to self-renew, proliferate and differentiate into multiple lineages and are usually radio- and chemoresistant. Therefore, these cells represent a novel therapeutic target, in particular for those malignancies which are refractory to conventional anticancer agents directed predominantly against cancer bulk populations, such as Glioblastoma Multiforme.

The role of altered cellular metabolism in Glioblastoma stem cells as a possible factor in tumour growth and recurrence is largely unknown, so characterization of new metabolic pathways adds valuable information on Glioblastoma stem cell maintenance and tumorigenicity.

Cancer stem cells often show characteristic changes in cell fatty acid metabolism. In particular, cell proliferation requires fatty acids for synthesis of membranes and signaling molecules. This paper deals with a very current topic and is aimed at increasing knowledge on the involvement of fatty acid and related enzyme metabolism as possible target of therapeutic agents against cancers. In addition the title and the abstract convey what has been bound.

Authors aimed to gain information on the involvement of ACSVL3 in the maintenance of glioblastoma cell stemness and the capacity of GBM-derived neurospheres to initiate tumor xenograft formation. As concern this aspect the question they posed is well defined.

This paper deals with a very important topic due to the role of fatty acid related ACSVL3 metabolism in modulating phenotype of cancer stem cells. In addition the effect of ACSVL3 loss-of-function which promotes cancer stem cell differentiation and inhibits their tumor-initiation properties is relevant in the aim to have more insight in studying stem-like cell response to new therapeutic treatments of cancer. However the used methodology mostly overlaps that reported in a previous work published by the same group (Acyl-CoA Synthetase
VL3 Knockdown Inhibits Human Glioma Cell Proliferation and Tumorigenicity Cancer Res 2009; 69: (24), 2009) with the exception of experiments related to differentiation processes induced in stem-like cells as response to ACSVL3 knockdown. For this reason, I suggest that a detailed description of the methodology has to be done only when dealing on new procedures. In the other cases methodology has to refer to literature as for now available.

The methods used are appropriate and well described. Experiments have been consistently performed according to what proposed in the abstract and results are comprehensive. Stem cancer cells before and after differentiation are checked for either surface and functional markers and tested for their tumorigenicity in vivo. Effects of ACSVL3 modulation of stem cell proliferation, differentiation, growth as neurosphere and efficiency in colony formation in soft agar are well described as well as the response of tumorigenicity in vivo.

Statistical analysis of the results sounds correctly performed.

The writing is good.

The only critical point of this paper is the Discussion that is poor and only built on reported literature data and on repeated Results section data. In particular, the authors demonstrated that the effect of induced differentiation was specific for ACSVL3 as shown in a comparison with ACSF2. Being ACSF2 a related acyl-CoA synthetase family member that activates medium-chain fatty acids, while ACSVL3 is strictly involved in fatty acid long chain elongation, possible hypotheses on the mechanisms or possible pathways by which the knockdown of ACSVL3, responsible of long chain fatty acid synthesis, influences glioblastoma stem cells tumorigenicity or differentiation are to be given.

Based on my general comments and due to the scientific interest of the topic, I recommend this paper for publication after a convincing revision of the Discussion.

Minor comments

Authors report in the first paragraph of Results

“ACSVL3 expression correlates inversely with differentiation of GBM stem cells”

…….“ Immunoblot analyses show that ACSVL3 expression…… was found to be relatively low in adherent GBM cell lines not enriched for stem-like cells (i.e. U373 and U87) (Fig. 1A). In contrast, ACSVL3 expression was elevated in HSR-GBM1A and HSR-GBM1B neurosphere cells (Fig. 1A)”…. 

Indeed, in this figure the presence of ACSVL3 can be well detectable even if at lower concentration with respect to GBM1A and GBM1B stem-like cells. Moreover, in their previous paper (Cancer Res 2009; 69: (24), 2009) the authors refer to the highly tumorigenic cell line U87 as expressing ACSVL3 at high levels. Instead, in the actual paper U87 cell line has been reported as a cell line where ACSVL3 expression was found to be relatively low.

I suggest to modify the sentence as follow:
……“Immunoblot analyses show that ACSVL3 expression…… was found to be, respectively, absent or lower in adherent GBM cell lines not enriched for stem-like cells, (i.e. U373 and U87) with respect to more elevated ACSVL3 expression in HSR-GBM1A and HSR-GBM1B neurosphere cells (Fig. 1A)”……