Reviewer's report

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

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Reviewer: Justin Lathia

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In this manuscript, Sun et al. interrogate the role of the lipid metabolism enzyme fatty acyl-CoA synthase VL3 (ACSVL3) on cancer stem cell (CSC) maintenance in glioblastoma (GBM). The authors build on their previous findings and show that elevated ACSVL3 is elevated in CSC culture conditions with knockdown inhibiting self-renewal and tumor initiation. Overall, this is well-executed study and the conclusions are supported by the data. However, there are additional studies required to clarify some key issues.

Major Compulsory Revisions

1. The authors attempt to demonstrate that ACSVL3 is higher in neurosphere cultures than serum cultured cell lines and while the data support this, in part, it would be useful to show ACSVL3 expression in neurospheres and differentiated cells as well as high passage cell lines in the same blot (Fig. 1A, show differentiated GBM1A and GBM1B on the same blot). The differentiation using TSA are also not very strong, is there a way all 3 methods can be quantified? This is not essential but would clarify the message.

2. The more useful demonstration of ACSVL3 expression would be to show in the same group of cells (not cells in different culture conditions) that the sphere-forming compartment (CSC marker positive cells) have elevated ACSVL3. This could be marker based enrichment from sphere cultures or xenograft tumors.

3. The differentiation data when ACSVL3 is knocked down is very strong. Adding an Immunoblot for Sox2 would strengthen the authors' claims as CD133 total expression is not completely informative for the CSC state. Also, while not essential, it may be worth showing which part of the gene each siRNA construct is directed towards in a schematic.

4. To evaluate if the major impact of ACSVL3 knockdown is differentiation, the authors should also evaluate cell death using a standard method (Annexin V, caspase 3/7 activity, cleaved caspase 3).

5. The data on sphere formation suggests a difference in self-renewal and this would be further strengthened by performing an in vitro limiting dilution assay to also calculate stem cell frequency in each condition.

6. The tumor initiation data is also very strong and it would be useful to show this as a Kaplan Meier survival plot with appropriate statistical analysis.
7. The changes in ACSVL3 as a result of EGF and HGF are interesting, do the levels of their receptors change when ACSVL3 is knocked down?

8. Throughout the manuscript, at least 4 terms are used to describe self-renewing cell populations (GBM stem-like cells, cancer stem cells, cancer stem-like cells, glioma stem cells). While there remains controversy and complexity within the field, 1 standard term should be used. The ideal term would be “cancer stem cell” and this could be used if the authors can demonstrate the expression differences in point 2 as well as provide references for the models used where a population of the cells were more tumorigenic than another population (possibly based on marker expression). Showing tumor initiation after sphere culture with no other comparison would not be appropriate. If this cannot be achieved, “cancer stem cell enriched neurospheres” would be an acceptable alternative.

9. The terminology of GBM and glioma are used interchangeably throughout the manuscript and should be revised to use 1 term only, although it is appreciate that there is overlap between terms.

10. Fig. 2 – The knockdown data was confirmed using qPCR (“change” is misspelled in panel 2A), it would be useful to show protein data via immunoblots.

Minor Essential Revisions

1. Abstract – The last sentence would read better if stated as follows “This study investigates the involvement of ACSVL3 in the stem cell maintenance of glioblastoma (GBM) and the capacity of CSCs (or cancer stem cell enriched neurospheres)…”

2. Abstract and Results – the authors should consider replacing the term “stemness” with “self-renewal” as this is more appropriate.

3. Conclusions – in the first sentence, “lipids” should be replaced by “Lipids.”

4. Fig. 2 – The GBM specimen used in panel A should be included directly on the figure.

5. Fig. 5 – The resolution of the immunoblots is low. This could be due to the format in which the file was downloaded. For final publication, the authors should ensure the figure is of appropriate resolution.

Level of interest: An article of outstanding merit and interest in its field

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