Reviewer's report

Title: Targeting SPARC inhibits cervical cancer cell growth and metastasis by lentivirus-mediated RNA interference

Version: 1 Date: 18 June 2012

Reviewer: Shanna Arnold

Reviewer's report:

Reviewer’s Report

- Major Compulsory Revisions

The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

1) Why use such a high (60) MOI, especially with a lentiviral shRNA construct?

2) What antibodies were used for the western blots? Please include them in the Materials and Methods/Western blot section.

3) In the Materials and Methods/Western blot section, it is stated that band intensities were analyzed. However, none of the figures include the densitometry analysis values. Please provide the % or fractional changes measured in all the western blots in figures 2, 3 and 7.

4) In Materials and Methods/MMP zymography section, please give the concentration of protein that was loaded in each well in addition to the 10ul volume. Where all samples loaded at equal concentrations? This is not clear from either the materials and methods or from the figure 7 legend. The reason this is important is because you show in figure 7a that MMP2 and MMP9 mRNA is dramatically reduced by knockdown of SPARC. The question is, does this translate to a change in protein expression as well as activity? The zymography has the capability of showing changes in MMP9 expression and “activity” by looking at the pro and active bands. Figure 7c is not showing the entire gel and only two bands are present. It seems that the gel was not run long enough to resolve the 4 bands (pro-MMP9, active MMP9, pro-MMP2, and active MMP2) that is generally seen by zymography. What are you trying to conclude from figure 7c? That MMP2 and 9 protein is reduced or that they have less activity after SPARC knockdown? You state “activity” in your results section but this zymogram does not support activity. As it stands now, figure 7c shows active MMP9 at 92kD and pro MMP2 at 72kD. The “pro” form only tells you expression data while the “active” form tells you activity at time of cell or tissue lysis. The point is, the decrease you see in the zymography band for pro MMP2 is most likely due to a decrease in protein level rather than activity.

5) In the next to the last paragraph in the discussion, the authors make the
statement “There must be another pathway about SPARC promoting cell-extracellular matrix adhesion” in response to not seeing changes in the membrane localization of integrins beta 1 and 3. However, this is an incorrect assumption. Although alternative pathways are always possible there is no proof in this manuscript that the effects of SPARC knockdown on adhesion is NOT integrin mediated. All the data suggests is that it is not a change in membrane localization of integrins beta 1 and 3. The authors would have to have done experiments revealing the “activity” of the integrins such as using antibodies specific to the active form of the integrin or colocalization with downstream mediators such as talin etc. I suggest removing this statement altogether and just focus on specifically what the data tells them. Lastly, during the sorting, were the cells permeabilized? The methods section makes it seem that the cells were not fixed and were not permeable. If this were the case, then the flow data would support membrane localization (or cell-surface expression) of the integrins and not the “expression” of the integrins as stated in the discussion. Please clarify and if it is on non-permeable cells change the results and discussion to reflect membrane localized or cell-surface expression of the integrins rather than just stating the “expression” of the integrins.

6) All line graphs in all figures containing them need to have either bigger symbols or different colors to delineate the different curves. It is very hard to tell which line/marker goes with what cell line.

7) Histology (H&Es) of the resected tumors in figure 1D,E and figure 6 is a necessity. This can be included as separate figures or as supplemental figures.

8) As stated above, figure 2B, 3B and 7B need densitometry measurements.

9) A table of lung metastases needs to be included in the manuscript. This table should include the incidence and events (or average lung colony size) etc. If space is limited, I suggest moving the RT-PCR primer table to supplemental.

- Minor Essential Revisions
The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

1) Title change to “Targeting SPARC by lentivirus-mediated RNA interference inhibits cervical cancer cell growth and metastasis” so that the prepositional phrase comes directly after what it is referring to rather than at the end.

2) In the first sentence of the methods section in the abstract, “cervical cancer cell line” is plural so it needs an “s” to make it “lines”.

3) There are many grammatical errors in sentence structure, comma placement and the misuse of conjunctions that should be addressed for improvement on readability.

4) Change “ovarian” to “cervical” in two locations in the methods section of the abstract.

5) “down-regulaed” in the results section of the abstract needs a “t”

6) Give details in Materials and Methods/Cell Lines on cells used. For example,
HeLa are HPV18 positive, 3 integrations, and cMyc driven. SiHa are HPV16 positive with 1-2 integrations. There is also data on the chromosome number that you might choose to include. HPV status and oncogene overexpression make a difference in how cervical cancer cell lines respond to genetic manipulation. There are several HPV negative cervical cancer cell lines that might respond differently than HPV positive lines.

7) In Materials and Methods/Isolation of subclones section, “10/ml” should be “10 cells/ml” for clarity. Also, “2~3” should be “2-3” weeks.

8) Figure 1E is dark and the resolution is fuzzy. Can this be corrected with imaging software or by reducing the image size?

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

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

**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