Reviewer’s report

Title: SUMOylation of sPRDM16 promotes leukemogenesis in acute myeloid leukemia

Version: 2
Date: 27 July 2015

Reviewer: Josephine Wixted

Reviewer’s report:

MCR: Major Compulsory Revision
MER: Minor Essential Revision
DR: Discretionary Revision

1. MCR. The use of “leukemogenesis” in the title (p1) and body of the paper does not accurately describe the process that was studied in the body of work. “Leukemogenesis” is defined as the process of tumor development at its earliest stages including initiation. The authors overexpressed sPRDM16 in the already established AML cell line THP-1. The results showing enhanced proliferation and decreased differentiation in this one cell line supports the idea that overexpression of sPRDM16 promotes AML progression not leukemogenesis.

2. MER. Abstract, p2. The use of “Ectopic overexpression” has not been supported by the figures presented. Based on the literature leukemic cells derived from AML patients exhibit enhanced expression of sPRDM16. The authors did not demonstrate whether sPRDM16 is already expressed in THP-1 cells (see comment 4). If sPRDM16 is expressed in THP-1 (at low or high levels of expression) then adding additional sPRDM16 by generating stable lines would not be considered ectopic expression – just overexpression.

3. DR. Introduction, p4. Line 55. Authors mentioned that the short isoform of PRDM16 (sPRDM16) has previously been shown to be SUMOylated. Please comment whether the long isoform is also SUMOylated.

4. MCR. Results, p11. The authors should demonstrate the protein expression of sPRDM16 in normal monocytes as well as in the THP-1 cell line before transduction. The authors should demonstrate protein expression of sPRDM16 in vector-THP-1 cells, sPRDM16-WT-THP1, and sPRDM16-K568R-THP-1 cells.

5. MER. Results, p11. Please briefly describe the cloning process and/or include a figure model of where FLAG was inserted into PRDM16 to generate only the short isoform of PRDM16.

6. MCR. Results, p11. The authors first describe the soft agar assay as an assay to measure self-renewal and later describe the assay as an assay to measure anchorage-independent growth. THP-1 cells are already anchorage independent in growth characteristics. Based on photographs presented it is hard to tell whether the number of colonies truly differ, however, the size of the colonies appear to be significantly different between the tested groups. Please measure
the size of the colonies as an indication of self-renewal and the number of colonies as an indication of anchorage independent growth. Please indicate the cut-off size used to determine “colony” and “not colony” in figure legends.

7. MER. Fig 1E and 3C. The flow experiment may be represented as a histogram as well as dot plot to demonstrate the level of CD11b expression in the whole population and compared to isotype control. This would aid the readers in observing the differences in the peaks and overall surface expression of CD11b in the whole population. As the data stands showing the dot blots, the % population that is “positive” for CD11b is dependent on an arbitrary marker set at 10 on both axes. Additionally, please state the statistical significance (p values) for all samples displayed.

8. MER. Under Experimental Procedures, p4. Please describe the method used to measure cell adherence as was used for Fig 1F and 3D.

9. MER. Results, p13, line 232. Please describe definition of “SUMOylated Sharp” if this is not a typo. Should this read “SUMOylated sPRDM16”?

10. DR. Results, p14, line 262. It may be best to say “reduced” rather than “partially abolished”.

11. MER. Figures. Figure 4B. The sPRDM16 and the K568R samples have overlapping error bars and therefore cannot demonstrate significant differences between the two.

12. MER. Results p15 and Figure 4F. The authors suggest that the “body weight loss in sPRDM16-WT-transplanted groups was more serious than that in sPRDM16-K568R-transplanted group and the Lenti-Vector-transplanted group”. This interpretation appears to be an overstatement of the results shown in Figure 4F. There does not appear to be any statistical difference between any of the groups tested.

13. MCR. The gene expression data is interesting, however, please provide a discussion on whether CD11b was analyzed in the dataset and why it was not one of the genes shown to be differentially expressed between the WT and K568R cells following PMA treatment.

14. MCR. Figure Legends. All Figures. Although the authors mention that their graphs represent 3 independent experiments for most experiments, there is no indication of how many replicates per experiment. For Fig 1C and Fig 3A, please indicate that the photographs are representative of (#) of plates per (#) of experiments.

15. MCR. The data is interesting, however, it would be much more convincing if more than one cell line was used in the study and if knockout experiments demonstrated that sPRDM16 was vital to THP-1, or AML, proliferation. It would aid the argument that SUMOylation of sPRDM16 aids tumor progression if an experiment was performed that knocked out endogenous sPRDM16 protein expression and rescued with sPRDM16-WT but not sPRDM16-K568R.

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: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.