Reviewer’s report

Title: Up-modulation of PLC-β2 reduces the number and malignancy of triple-negative breast tumor cells with a CD133+/EpCAM+ phenotype: a promising target for preventing progression of TNBC

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Reviewer: Samantha Oakes

Reviewer's report:

The study by Brugnoli and colleagues is a very simple and largely descriptive study of the properties of a possibly previously undiscovered small subpopulation of MDA-MB-231 cells characterised by high expression of EpCAM and CD133, which appear to have increased proliferation and invasiveness determined using a single type of real time cell assay. The expression of phosphoinositide-dependent phospholipase C (PLC-beta2), which the authors have previously shown to reduce the expression of CD133 and invasiveness of MM231 and MM468 TN breast cancer cells, inversely correlates with the expression of CD133 as previously shown and appears to be lowest in the EpCAM/CD133 positive population. Further forced expression of PLC-beta2 appears to reduce the expression of CD133 and EpCAM in this population and was associated with reduced invasiveness and proliferation. The final piece of evidence (that appears to be only performed once) indicates that forced expression of PLC-beta2 in unsorted MDA-MB-231 cell lines reduced the proportion of CD133/EpCAM positive cells and potentially results in an increase in a very small number of CD44 positive CD133 neg and EpCAM positive cells.

There a a number of concerns that need to be addressed and the manuscript could be substantial improved with the addition of a number of additional experiments:

1. Although it has previously been observed that EpCAM expression is low to absent in MDA-MB-231 cells (BMC Cancer201212:501 DOI: 10.1186/1471-2407-12-501) here the authors comment that EpCAM is relatively low as indicted in Figure 1A. Unfortunately it is difficult to determined whether this relative level is a result of the low expression of EpCAM in MDA-MB-231s or as a result of the techniques and gating strategies used in the current study. To make this comment, the authors need to compare the expression (and proliferation and invasive properties) of EpCAM and CD133 (which is normally high in MDA-MB-231 cells (Liu et al 2013 Oncogene 32, 544-553) to other cell lines such as T47D/MCF7s and 468s and to show that the staining and gating strategy is able to detect high levels of these antigens. This is particularly important given that the combination of EpCAM and CD133 does not result in obvious populations on FACS analysis and that the MACs separated
CD133+ populations have such low purity (<50% purity on Figure 1C). Without this data the EpCAM/CD133 enrichment strategy is just not convincing.

2. On the poor purity of EpCAM+/− and CD133+ populations, given there are no defined populations, a double sorting strategy may yield substantially better results. Is the expression of these antigens stable within these populations over time?

3. Figures 1D and E, it would be preferable to have additional and complimentary measures of proliferation and invasiveness and these should be compared to cell lines with lower proliferation and invasiveness e.g. 468 and MCF7.

4. Figure 1E. What does the significance indicate? were t-tests performed between populations or were the CD133− and CD133+ populations combined (independent of EpCAM expression) to reach significance. This results reveal nothing more than previously demonstrated that CD133 high expressing cells have greater invasive capacity. Further enrichment with EpCAM reveals nothing more.

5. Figure 2A. Although PLC-B2 appears to inversely correlate with expression of CD133 and EpCAM, double immunofluorescence of PLC-B2 with EpCAM and CD133 would indicated whether this occurs on a cellular basis or in a global fashion on the entire cellular population

6. Figure 3. The effects of siRNA and forced expression of PLC-beta2 in a parental population of MDA-MB-231 cells appears to have been done only once. There are no error bars in this figure to indicate that multiple independent experiments have been performed. Until this is provided, none of the data indicating the effects of PLC-beta2 on population stratification using EpCAM/CD133 can be interpreted. Further the small increase in number of CD44/CD133- and EpCAM + cells.

7. What happens when PLC-beta2 is reduced by siRNA in a non-invasive cell line such as MM468s and T47Ds, does the expression of CD133 and EpCAM increase nd does this increase proliferation and invasiveness? And conversely what happens with forced expression of PLC-beta2 in invasive CD133+ subpopulations from other cell lines.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
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No

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