Reviewer's report

Title: Essential microRNAs for self-renewal of breast cancer stem cells initiating from the counterparts of mammary epithelium

Version: 3
Date: 16 March 2015
Reviewer: Qun Zhou

Reviewer's report:

Major Comments:

1. Fig. 1B: It would be beneficial to verify myoepithelial and luminal colonies using markers such as p63 or cytokeratin 14 (for myoepithelial) and cytokeratins 7/8 (for luminal) respectively. The differentiation data for the MCF-10A population excluding MUC1-ESA+ cells is a necessary negative control for definition of MUC1-ESA+ as a stem cell population, and should be included in the figure. Staining of this population with the same markers as detailed above should be done as negative control. Additionally, Day 0 isolated MUC1-ESA+ cells should be stained and compared with Day 10, to properly demonstrate that this population has stemness properties.

2. Fig. 1: It would be useful to the reader to include a graph of the colony frequency in addition to specifying the numbers in the results section.

3. Fig 2: The authors say that they had an 80% success rate in miRNA identification, verified by comparing their microarray and qRT-PCR data. It would be beneficial to provide these data to the reader.

4. As MCF10A and MCF7 are cultured cell lines of different genetic backgrounds, it is problematic to compare miRNA expression between them. It is important to verify these findings in a 3rd, MCF10A-derived tumor cell model, such as MCF10DCIS.

3. Fig 3D: Quantification of tumor volume in graph form is necessary. It is difficult to see tumors in the picture provided, and this is an ineffective method of tumor growth data.

4. Fig 3: Does miR-200c have similar effects at physiologically relevant levels of expression? It is possible that overexpression to such a high extent (300-400x) would have off target effects. miR-200c should be knocked down, in order to demonstrate increased self-renewal compared to untransfected cells that have endogenous levels of miR-200c.

5. Fig. 3: The authors should remove the tumors and perform in vitro assays for self-renewal to demonstrate that miR-200c has the believed effect. Immunofluorescence or qRT-PCR for stem cell markers would determine if miR-200c has an effect on the prevalence of the stem cell population in these tumors. Cells could be isolated from the tumors and used in mammosphere
assays to demonstrate that miR-200c has a similar effect in vivo to that shown in vitro in fig. 2B and C.

Minor Comments

1. The manuscript needs editing for English word and grammar use.

2. All microscopy pictures should include scale bars and the magnification the picture was taken at.

3. The manuscript should include a reference (Eur J Gynaecol Oncol. 2014;35(6):696-700) which previously verified PDCD10 as a target of miR-200c in MDA-MB-231 breast cancer cell lines.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: N/A