Reviewer's report

**Title:** Essential role of miR-200c in regulating self-renewal of breast cancer stem cells initiating from the counterparts of mammary epithelium

**Version:** 5  **Date:** 5 May 2015

**Reviewer:** Ravindresh Chhabra

**Reviewer's report:**

There is marked improvement in the revised manuscript by Sun et al. Most of the questions raised in my earlier review have been answered in the revised manuscript but I have few comments before the article is accepted for publication.

**Major compulsory revisions:**
1. The authors found miR-200c upregulated in BCSCs when compared with MaSCs and then go on to show that ectopic expression of miR-200c suppressed the tumorogenic ability. Does this mean MaSCs are more tumorogenic than BCSCs? Or does it mean that miR-200c is only associated with self-renewal properties and not with tumor initiating capability?
2. Scale bar should be included in microscopy pics
3. The authors need to give appropriate references to support their statement “It is hypothesized that BCSCs initiate from accumulating mutations of MaSCs”.

**Minor compulsory revisions:**
1. In the abstract section, what do the authors mean by “typical miRNA”
2. In the abstract section, under “results” subheading, the authors are claiming that the expression of miR-200c is lower in BCSCs than in MaSCs but they are contradicting this in results section under subheading “miRNA profile of BCSCs distinct from MaSCs”
3. In the methods section, under subheading “Microarray fabrication and miRNA hybridization”, the authors should provide GEO accession number for their submitted data.
4. In the results section, under subheading “Verification of miRNA microarray by qRT-PCR assay”, the statement “which were slightly downregulated in BCSCs and significantly upregulated in BCSCs” needs to be rephrased.
5. In the results section, under subheading “Verification of miRNA microarray by qRT-PCR assay”, the authors need to rephrase this statement “miRNAs with the consecutive change from MaSCs to BCSCs”. What do they mean by consecutive change?
6. In addition to miR-21 and -200c, miR-373* could also be a candidate as its expression decreases from MaSC to BCSC and further decreases in MCF7 when
compared with BCSC. It follows an opposite pattern to miR-21 and -200c
7. In the results section, under subheading “Role of miR-200c in regulating stemness of BCSCs and MaSCs”, correct the spelling of antagomir.
8. Fig 3d, it is difficult to make out the tumors in these pics. If possible, replace them with pics which show a clear view of the tumor.
9. The authors have throughout stressed that BCSCs are derived from MaSCs. This is one of the hypothesis but BCSCs may have an alternate source of origin. In my opinion, the authors should include this in the background section.

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

**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