**Reviewer's report**

**Title:** Expression of miR-34c induces G2/M cell cycle arrest in breast cancer cells

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**Reviewer:** Delia Mezzanzanica

**Reviewer's report:**

Achari and co-authors are presenting data supporting a tumor suppressing role of miR-34c in breast cancer mainly mediated by suppression of cell proliferation, due to induction of a G2/M cell cycle arrest, and by increase of cell death in a PKC-alpha independent way.

The paper is well written but the conclusion that CDC23 down-modulation can be responsible for miR-34c-induced cell cycle arrest is not fully sustained by experimental data. Furthermore, no mechanistic effect are provided for the pro-apoptotic role of miR-34c although in discussion the authors speculated in a possible down-modulation of pro-survival molecules.

**Major Compulsory Revisions:**

- In Figure 3, representative cell cycle profiles (G1-S-G2/M and sub-G1) should be shown for each cell line.
- Results related to Annexin V binding (Figure 4) should be shown together with simultaneous PI staining of live cells as bi-parametric dot plots.
- Although CDC23 3'UTR has been predicted to contain a putative miR-34c binding site by five different algorithms, a clear demonstration of miR-34c binding to CDC23 3'UTR is lacking. Furthermore, a demonstration that CDC23 silencing photocopies miR-34c transfection is also lacking.
- Data on PRKCA as possible target of miR-34c are not convincing: no effects are observed at protein level after miR-34c transfection and a significant downmodulation of PRKCA mRNA is observed only in one cell line (Figure 5). In one cell line (MDA-MB-468) PRKCA mRNA apparently increases in a significant way after miR-34c transfection in contrast with miR regulatory role.
- No data are provided about effects of miR-34c transfection on CDK4 and CDK6 mRNA expression levels.

**Minor essential revisions:**

- Is there any available reference for breast cancer TCGA data?
- It is not very clear what “new tumor events” means in Figure 1 G-I and in the corresponding results section. Is this end-point referred to tumor relapse and therefore Kaplan-Meier curves referred to relapse-free survival?
- It is not clear how many experiments are reported in Figure 2, (n=3) is referred to number of replicates of number of experiments?
Level of interest: An article of importance in its field

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’