Reviewer's report

Title: Kif2a Silencing Inhibits the Proliferation and Migration of Breast Cancer Cells and Correlates with Unfavorable Prognosis in Breast Cancer

Major Compulsory Revisions:

In this submission, the authors assess the expression of Kif2a in primary breast cancer tumors, and correlate expression with poor prognosis in breast cancer patients. The authors also assessed the role of Kif2a knockdown in breast cancer cell lines on proliferation, migration and invasion in vitro.

Overall this study is interesting, and does add to the knowledge of the role of Kif2a in the progression and prognosis of breast cancer. However there are a number of issues, including missing experimental details, omitted data, and over-interpretation of experimental results based on the data presented. These missing details need to be addressed before this manuscript is suitable for publication. These issues are outlined below:

1. The authors did not specify how the patient tumors were staged or graded, or who performed that determination. Was a pathologist involved in reviewing the cases for inclusion in the study? If so, the role of this expert should be acknowledged in the manuscript. Also, how was the patient data reviewed to ensure that only patients with complete follow-up (overall survival times) were included in the study?

2. The authors also describe performing IHC on patient tumor slides. How were the slides/tissue sections selected for inclusion in the study? What were the criteria (% viable tumor cells, presence of adjacent normal tissue, the number of tissue sections stained/patient) on which the authors based the selection of tissue sections for staining? Was a pathologist involved in the selection of the tissue sections?

3. With respect to the IHC staining, representative images of the different intensity categories for the Kif2a staining should be shown as a reference in the supplementary materials section. The pathologist involved in scoring the IHC sections should also be acknowledged in this study.

4. Details are lacking on many experimental procedures, including:
   a) Reference to cell culture procedures is not included;
b) How much total protein was loaded onto the protein gels;

c) GAPDH antibody dilutions, antibody supplier information, antibody incubation times, and how the chemiluminescence signal was detected (film, scanner?);

d) How the protein signal intensity was quantified (image analysis software), and how many replicates were included in this analysis;

e) It is unclear whether the authors performed real-time PCR of Kif2a expression, or RT-PCR and gel electrophoresis. Please clarify. If real-time PCR was performed, details on the methodology (SYBR green or TaqMan probes?), or how the mRNA expression level was calculated (fold change, or intensity levels?) need to be specified. If RT-PCR and gel electrophoresis were performed, how was the intensity of the bands determined?;

f) P-value in Figure 1B cannot be 0 – please include a number.

g) Many figures do not have a P-value stated either in the figure, or the figure legend;

h) No scale bars are included in any of the figures to indicate that the images shown were taken at the same magnification. Especially for Figure 2, where A-B, C-D, and E-F look like they were all taken at different magnifications;

i) The images in Figure 2 E and F are of very poor quality, and it is virtually impossible to see the FISH probe signals.

j) Figure 5 was provided in a very poor quality format - the reviewer could therefore not appropriately evaluate the data presented in this figure;

k) Details are missing on how the cells were counted for the migration/invasion assays – how many fields were counted within a filter? How many replicates were included in the experiment? Were the authors blinded?;

l) images of migration assays need to be included.

5. The authors state that two siRNA molecules targeting Kif2a were employed in their analyses, giving the same results. However the authors chose to only show the data for one siRNA molecule. In order to strengthen their postulate, the authors should show the data on both siRNA molecules (perhaps as supplementary data), as this would further support their conclusions of the effect seen in response to Kiff2a knockdown.

6. The authors state that Kif2a expression correlated with poor prognosis in breast cancer patients. The only data shown to support this postulate are the Kaplan-Meier curves in Figure 3, and Odds ratios stated in the text. However, this data is poorly described, and insufficiently analysed to support the authors’ conclusions. The authors should present a graph showing the Kif2a IHC expression levels of each patient, and indicate where the cutoff was chosen for the dichotomization between the high and low expression groups, along with the statistical analyses. The results from the prognostic analyses (including univariate, multivariate, and correlational analyses with clinical variables vs. months survival and survival rates between groups, odds ratios or hazards ratios, and correlation coefficients with confidence intervals and associated P values)
should be included in a table format in the results section of the manuscript.

**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:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests