Reviewer’s report

Title: Estrogen receptor (ER) signaling regulates the expression of the breast tumor kinase (BRK) in breast cancer cells

Version: 1 Date: 30 Aug 2018

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER COMMENTS: To view the full report from the academic peer reviewer, please see the attached file.

REVIEWER COMMENTS FROM REPORT: The overall objective of the manuscript is made clear right from the beginning which is great. Background section is drafted very well.

In the Methods section, I suggest that the authors provide a listing of all cell lines, as opposed to mentioning 'all breast cancer cell lines…'. The section should also include culture conditions etc.

'Stable cell preparation and Transfection' should ideally be 'Transfection and Stable cell line preparation' because transfection precedes selection for stably transfected cells. This subsection should also briefly describe how the stable cells were selected. I understand that reference to earlier publication(s) is provided but same basic details should be provided right here.

The authors mention that plasmids were gift from Dr. Michael Mancini. I suggest that the Institution details of Dr. Michael Mancini should be provided.

Are there any specific identifiers for the 24 tumor samples the data for which was downloaded from TCGA?

The very first results are assessment of BRK levels in 24 patients (TCGA). The authors look at samples that represent different human cancers. Are the observations statistically reliable, given that there are just a few samples representing individual cancer types? Also, since the samples represent different cancers, in this reviewer's opinion it is not appropriate to describe results in the way they have been presented. For example, instead of mentioning that 15 of the 24 cancer had higher BRK, they should mention which cancers had high levels, which cancers had moderate levels and which ones had lower levels, compared to normal. Irrespective of outcome, this is not a very convincing piece of data, given the sample size.

In continuation of my above comment, it is possible that the authors have not presented their case very well. Instead of 24 patients, what they actually meant was perhaps 24 different human cancers. If that is the case, the results are good! But the authors need to make it explicitly clear
that they are talking about analyses of 24 different cancers and not 24 patients!! Please note that the description in Methods definitely seems to suggest that authors are talking about 24 samples (patients). They need to re-phrase sentences to make this very clear.

When checking for the correlation of BRK with breast cancer subtypes, the authors seem to convey a message that BRK levels were higher in ER+ breast cancers, compared to HER2+ or TNBCs. In this reviewer's opinion, the comparison should be made with normal controls as the primary analyses. The comparison between subtypes is also informative but the diagnostic value can only be assessed by comparing levels with normal tissues.

In the Results section 'BRK protein expression correlates with tumor progression' the authors state in the very first sentence that their intent was to corroborate BRK at protein levels so as confirm mRNA results. This is not correct. The intent of the results presented in the section was actually to correlate BRK levels with tumor metastasis and progression (as rightly mentioned in section title). I would suggest a careful editing of all the text to minimize such errors and improve presentation.

The heading 'BRK protein expression is generally higher in ER-positive breast carcinomas compared to other subtypes' does not entirely reflect the contents. The real goal of the analyses in this section is to establish that metastatic samples from ER+ breast cancers have higher BRK protein levels than metastatic samples representing other breast cancer subtypes.

The data from cell lines is too dramatic and seems to suggest that ER+ cells have higher levels than all other cells. Is this also the case with human samples? Do all breast cancer patients with ER+ disease have higher BRK levels than HER2+ and TNBCs?

I commend the authors that they have provided a lot of information and data. However, this is not accurately and adequately reflected in the Abstract. I recommend that the authors re-write the Abstract to reflect on all the contents and to make it more impressive.

Finally, a tight editing for the manuscript is needed. I have pointed out some concerns above. Additionally, improvements are needed in the language / grammar at many points. A few examples are - Please remove 'any' from this sentence in the Abstract - 'Herein, we investigated the correlation of BRK with any breast cancer subtypes….' and the sentence 'As shown in Figure 1B, the log2 fold change of the BRK mRNA in different subtypes of breast cancers' is not grammatically correct.

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**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?
If not, please explain in your comments to the authors.
Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
Not relevant to this manuscript

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