Reviewer’s report

Title: ERα-related chromothripsis enhances concordant gene transcription on chromosome 17q11.1-q24.1 in luminal breast cancer

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Reviewer: Noriko Saito

Reviewer’s report:

General Comments:
In this manuscript, the authors focused on concordant transcriptional upregulation of genes on chromosome 17q11.1-q24.1, in breast cancer cells. They found that the upregulation may be due to chromothripsis that brings genomic rearrangements between ATC loci and ER (ESR1) hub. They identified that one of the gene in the ATC locus, TLK2 is a tumorigenic driver for aggressive luminal breast cancer. They also showed that the TLK2 inhibition induced anti-proliferative effect in vitro and in vivo. This is an interesting work, covering from bioinformatic analyses through identification of the therapeutic target. The manuscript is clearly written. However, there are a few points that need clarification.

Major comments:
1. The authors should correctly use the term "ERα", not "ESR1". ESR1 is a gene name that encodes the ERα protein. Please see: https://www.uniprot.org/uniprot/P03372, for example. When the authors mention "ESR1 sites", they probably mean "ERα binding sites". Likewise, "ESR1 hub" should be "ERα hub". They have to correct "ESR1" to ERα, throughout the manuscript, including the title, unless they truly mean the gene name.

2. Is it possible that the mutation in ER is associated with ER-mediated chromothripsis (Fig.3d)? I am wondering whether ER mutations simply increase the gene expression in the five regions at 17q (Fig.2b).

3. The authors claimed that TLK2 KD (siTLK2) was sufficient to inhibit cell growth, by comparing with TLK2 KD plus PPH treatment (siTLK2+PPH). Considering that PPH is the TLK2 inhibitor, they should explain why the PPH treatment only (SiCtrl+PPH) shows very little effect on cell proliferation (Fig. 5f). What was the concentration of PPH in Fig. 5d and f?

4. The authors proposed that TLK2 is an actionable target for luminal-HER2 subtype, and the TLK2 inhibition therapy is effective to add the anti-HER2 therapies. However, the correlation between TLK2 and HER2 or luminal-HER2 cancers is not clear. The authors need to determine whether TLK2 is also a tumorigenic driver in HER2 or luminal HER2 cancers, and whether its inhibition has anti-proliferative effect.

Minor comments:
1. It would be better to define the genomic region A to E in the text.

2. It will be helpful for understanding the importance of transcription, if the authors add the RNA-
seq and ChIP-seq data of RNA polymerase II in Fig.4a.

3. It is recommended that the abbreviation for thioridazine be unified as "TRD" in Figure 5c or "TRZ" in the text.

4. Please clarify the difference between the genome interaction and the genome rearrangement. The authors should avoid using "interaction", when they mean genome rearrangement, in lines 22 and 24 on page 13, for example. The genomes are physically interacted in the nucleus, without changes in the DNA sequences. The genome interactions are generally detected by C (chromosome conformation capture)-technologies.

5. The authors should rename the "ESR1 sites". It is not clear if they simply represent a cluster of ERE (estrogen receptor responsible element) motifs, or they are proven to be bound by ER by ChIP-Seq experiments, for example.

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.

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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