Reviewer's report

Title: Transcriptomic and ChIP-sequence interrogation of EGFR signaling in HER2+ breast cancer cells reveals a dynamic chromatin landscape and S100 genes as targets

Version: 0 Date: 10 Oct 2018

Reviewer: Daniele Lecis

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In the manuscript "Transcriptomic and ChIP-sequence interrogation of EGFR signaling in HER2+ breast cancer cells reveals a dynamic chromatin landscape and S100 genes as targets" by Nava M. and colleagues, the authors investigate the effect of EGFR stimulation on gene transcription in order to identify possible determinants of anti-Her2 therapy resistance. Starting from the notion that EGFR is upregulated in HER2+ breast cancer cells resistant to trastuzumab, the authors stimulated the HER2 positive breast cancer cell line SKBR3 with EGF in time-course experiments and performed extensive RNA-sequence and ChIP-sequence for H3K18ac and H3K27ac. With this approach, they identify clusters of genes which are activated or repressed at different timepoints. Some of these genes are discussed and their possible implication in cancer progression and resistance to therapy is described. Several mechanisms can hinder the efficacy of trastuzumab, but the authors decide to focus on the capacity of EGFR to promote the expression of genes which could affect the treatment. This work is clinically relevant and present new data that could be instrumental for future validation.

In my opinion, this work presents a few limitations that should be considered before publication:

1) Only one cell line is employed (SKBR3) for all the experiments and therefore findings can hardly be broadened to other HER2 positive cancer cells.

2) Genes identified as affected by EGF and discussed as crucial are not validated by qPCR and/or western blot.

3) No functional experiment is shown to verify the involvement of the identified genes in trastuzumab resistance. This represents a drawback since the authors state several times that the aim of this study is to identify possible EGFR-mediated mechanisms of resistance to trastuzumab (Lines 48-50, 369-370, 459-462).
Minor points

1) In Figure 2c, there is not enough space for the name of the identified pathway making it difficult to appreciate the findings (e.g. positive reg of...)

2) In Figure 3a, the authors find accumulation of H3K18ac and H3K27ac also in genes repressed (CPNE1, MAX). If I understood correctly, these should be marks of activation. Probably this should be discussed more extensively.

3) The authors study the EGF-dependent gene expression also at very late timepoints, contrarily to what reported in literature (lines 136-137). Nevertheless, it should be considered that at late timepoints, the effect observed could be due to the activation of other pathways activated in turn (e.g. by cytokines or soluble factors released in an EGFR-dependent manner) and not merely specific to EGF stimulation.

4) The importance of p21 (lines 210-214) and S100 genes (lines 357-362) is speculated but not experimentally tested.

5) In general, the manuscript lists a number of genes affected by EGF stimulation. Probably it would be possible to summarize everything in a table and reduce the text, hence making the paper easier to read.

6) It would be extremely interesting, but I understand it is not the aim of this work, to compare the effect of EGF with the other ligands which stimulate EGFR (e.g. TGFa). What do the authors expect?

**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
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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