Reviewer’s report

Title: Overexpression of CD44 accompanies acquired tamoxifen resistance and augments cellular sensitivity to the stromal factors, heregulin and hyaluronan.

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Reviewer: Pamela Klingbeil

Reviewer’s report:

General remarks
The manuscript addresses the interesting finding that the adhesion molecule CD44 is over-expressed in tamoxifen resistant MCF7 cells compared to the drug sensitive counterpart. The authors correlate association of CD44 with members of the erbB-family with the aggressive phenotype of these cells and can demonstrate a CD44-dependence of the erbB ligand Heregulin induced migration, invasion and proliferation. They also demonstrate CD44-ligand Hyaluronan induced migration in TamR cells as well as an effect of hyaluronan on erbB dimer composition.

This is reasonable study with some interesting findings. Regrettably, the overall appearance is quite sloppy (missing data, spelling mistakes, out of focus images) and the described findings do not always reflect the presented data very well. In order to not generalize the findings the title should mention the MCF7 system that is described. A functional link between CD44 over expression and tamoxifen resistance was not addressed, which would have been interesting. Taken together, the manuscript needs revision on different levels.

While CD44-dependent activation of EGFR is adequately demonstrated, the data for CD44 involvement in erbB2 activation is less convincing (erbB3 activation was not addressed). This limits the information content of the paper. The authors provide clear evidence for a CD44-HA-dependent activation of EGFR and downstream MAPK signalling and for a switch in HA-induced erbB dimer composition in TamR MCF7 cells.

Discretionary Revisions
1. When MCF7 cells and TamR MCF7 cells are compared, it would be advisable to keep the control cells in the same charcoal stripped serum as the TamR cells and only add estrogen. This would exclude effects of growth factors in the foetal calf serum.
2. The figures could be grouped differently for improved clarity and readability and to avoid presenting similar data twice (Fig. 3B and 4D upper panel).
3. Figure 6ABCD can be omitted and the data only described in the text.
4. Supplementary table 1 does not give any additional information; instead it is partly misleading and should be omitted.
5. Loading controls for western blots should be done on the same blot (this does not seem to be the case in all blots).

6. Labelling of data should be more coherent throughout the manuscript (e.g. the antibodies used for western blot detection should be indicated in all figures like in Fig. 1, molecular weights should be indicated in all figures or in none).

Minor essential revisions

1. Spelling mistakes need to be corrected.
2. Figure 2 should be omitted as it does not provide any information about co-localization. It only demonstrates localization of CD44 and erbB2 in the plasma membrane (confocal images are needed to make assumptions about co-localisation). The erbB3 staining looks like nucleoli localisation or might be unspecific. If the authors want to demonstrate nucleoli localisation this should be discussed in the text. Fig 2B is missing but described in the figure legend.
3. Fig. 5B, C, D: how are the control cells treated in the functional assays (untreated, NTsiRNA treated)?
4. Fig. 8: HA treatment leads to a loss of EGFR:erbB3 and an increase of erbB2:erbB3 dimerisation not the other way round as stated in the legend.
5. Fig. 7A: Non-HA treated cells should be used as reference here to show HA-induced migration and the effect of the inhibitors on the HA-induced migration. Data for MAPK inhibitor is missing, but described in the legend and should be shown.

6. Materials and Methods:
   • The lysis conditions (detergents) used for the IP experiments needs to be mentioned.
   • Inhibitor concentrations have to be mentioned.

7. Statistical testing is missing in Fig. 6E and Fig. 7B. p values should be shown for all statistical tests.

Major essential revisions

1. A second CD44 siRNA should be used to ensure specificity of the observed functional phenotype (was a single siRNA or a pool used for the treatment?)
2. Fig. 3C: the finding that CD44 k.d. inhibits erbB2 phosphorylation can not be seen in the presented western blots due to underloading (or down regulation) of total erbB2). This should be reflected in the text.
3. Fig 5A: inhibition of Hrg-induced erbB2 phosphorylation due to the CD44 k.d. is minimal. No inhibition of MAPK phosphorylation is visible. Only EGFR and AKT activation is convincingly linked to CD44 expression in this figure, which should be adequately described in the text.
4. Fig. 6F is missing but described in the legend and needs to be shown.
5. Fig. 7A: the legend states that HA treatment of TamR cells leads to activation of EGFR, the blot does not show this. HA leads to activation of erbB2 in this blot,
but CD44si treatment does not abrogate this. The described effect can only be seen for MAPK activity; the text should describe this adequately.

6. Fig. 8: Immunoprecipitation data: To demonstrate molecular associations, IPs should be carried out for both association partners to ensure specificity.

• Her2 detection in EGFR-IP is summarised in supplementary table 1, but the data is not shown anywhere and should be included.

• The use of a CD44-antibody for IP is mentioned in the methods and legend, but the data is not shown. Detection of erbB2 and erbB3 protein in CD44-IPs should be demonstrated.

• IgG-control IPs should be included in the figure.

Level of interest: An article whose findings are important to those with closely related research interests

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