Reviewer's report

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

Version: 1 Date: 8 February 2012

Reviewer: Ivan Plaza-Menacho

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In this study Hiscox et al claim that in TamR cells over-expressing CD44, which directly interacts with erbB2, siRNA-mediated suppression of CD44 significantly attenuated their response to heregulin, inhibiting heregulin-induced cell migration and invasion. Furthermore, TamR cells exhibited enhanced sensitivity to HA treatment resulting in modulation of erbB2 dimerisation, activation of erbB2 and EGFR and induction of cell migration. Overexpression of CD44 in MCF7 cells, which lack endogenous CD44, generated an HA-sensitive phenotype, with HA stimulation promoting erbB/EGFR activation and migration. They conclude that CD44 may present an important determinant of breast cancer progression in the setting of endocrine resistance.

This is an interesting study aiming to give insights into an important clinical problem: resistance to endocrine therapy, in particular to Tamoxifen. However in order to assure publication some experiments are needed to strength the impact of the study.

Major comments

Despite the appealing title of the article there is no data in the whole study that proves the correlation of CD44 expression and Tamoxifen resistance. What is the effect of siCD44 in TamR cells in response to the drug? Are MCF7 cells over-expressing CD44 resistant to Tamoxifen? Besides, no clinical evidence of their findings in patient material is shown either.

Fig.1 Validation of their findings in tumor samples from patients is recommended to increase impact of the findings. Is CD44 over-expressed in Tamoxifen resistant tumour samples?

Fig.2 Normal MCF7 cells staining as control should be shown side by side to Tamoxifen resistance cells.

Fig.3 Why the effect of siCD44 is stronger in EGFR phosphorylation than in erbB2 phosphorylation when comparing total levels of protein.

Fig.6 Western blots are not convincing. What is the effect of siCD44 in response to tamoxifen?
Blots showing pERK1/2 increase upon HA stimulation of CDC44 are not convincing.

Alternative assay to show increase migration should be shown, wound closure experiments are difficult to control and there are available more elegant and better ways to assess cell migration.

Fig. 7 Quantitation of WBs is necessary for conclusion, in particular ErBb2 phosphorylation (when said activated, which phosphorylated residue is activated).

An alternative experiment for wound closure is recommended.

Fig. 8 The quality of the blots, in particular left panel are not of a good quality.

Fig. 9 Quantitation of the Wbs should be considered.

Minor comments

The Western blots are not properly align in many of the figures.

The resolution of panel A and C in figure 6 is not good enough.