Reviewer's report

Title: Localization of phosphorylated ErbB2-4 and heregulin in colorectal cancer

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Reviewer: Cornelis Sier

Reviewer's report:

The manuscript by Mitsui and co-authors describes a basically immunohistochemical staining study of heregulin and ErbB receptors and their phosphorylated forms in 155 tumor samples from colorectal cancer patients, complemented by expression data obtained from colon cancer cell line of the same proteins. The subject is not very original but because data are available for a complete family of receptors plus ligand it is certainly interesting but for a limited audience. The methods are classic, relatively well described, and the data are adequately discussed. The number of patients does not allow thorough multivariate statistical analyses, especially because colon and rectum tumors could/should be considered as different tumors for survival prognosis. The uni/multivariate Cox analysis model seems awkward. Why are tumor grade or location (colon/rectum) not integrated? Because of the subgroup ratio’s (e.g. pERbB4 25 vs 130) strong statistical significances (P=0.001) should be considered indicative not conclusive. All in all, the conclusion of the authors that this study is indicative, but that further investigation is warranted is valid. Although the paper is clearly written it needs minor grammar corrections. Considering the limited message of the paper, it could be more compact, especially the introduction and discussion.

Minor points:

* Introduce heregulin better in the first paragraph of the abstract.
* The choice for HCT116 is described, but for DLD1 and Caco2 not. Discuss and explain the different results between the cell lines. Figure 1A should contain HCT116 as well. Because of the similar MWs, describe for this figure whether 1 blot is re-probed (plus striping conditions) or whether separate gels are used for each antibody.
* Indicate why 100 ng/ml HRG was used (response curve?) and incubation time(s).
* Indicate for the non-p antibodies which domains (extra/intracellular) are targeted.
* Indicate the sample period for the patients.
* Indicate kappa statistics between observers.
* Indicate in Table 4 and Table 5 how many patients are analyzed in total (155/95) and how many are in the groups.
* Figure 1, Indicate magnifications and put higher magnification inserts and
arrows where multiple forms are indicated.

*Figure 2, change pErBB3 into pErbB3

* Table 1, correct polyclonal, rabbit IgG = monoclonal?

* Table 4, officially Depth is not a continuous variable (numeric)

Minor essential revisions

I declare that I have no competing interests