Reviewer's report

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

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Reviewer: christopher daly

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In this manuscript the authors examine the subcellular localization of phosphorylated ErbB2, ErbB3 and ErbB4 in cultured colorectal cancer cells following stimulation with heregulin. In addition, the authors use immunohistochemistry to assess the expression of heregulin and ErbB proteins in a series of colorectal cancer specimens and attempt to determine whether any of these proteins has prognostic value.

Compulsory revisions

In Figure 1, the authors stimulate cultured colorectal cancer cells with heregulin, fractionate the cells and then assess the distribution of phosphorylated ErbB2, ErbB3 and ErbB4 in the cell fractions. The authors conclude that p-ErbB2 and p-ErbB3 (but not p-ErbB4) can localize to the nucleus following stimulation. However, the methodology is poorly described. Were the nuclear fractions analyzed by immunoprecipitation/western blotting or just by straight western blot? The Methods section implies that the nuclear fractions were used directly in western blots. If this is the case, how was the phosphorylation status of ErbB2 and ErbB3 established, since the western blot antibodies are against total ErbB2 and ErbB3? This should be clarified in the Methods section.

Importantly, the authors do not demonstrate the purity of their cell fractions by western blotting for known nuclear/cytoplasmic proteins. This is an essential control.

In addition, it is unclear what percentage of p-ErbB2 and p-ErbB3 are in the nuclear fraction? The western blot in Fig. 1A seems to suggest that ~50% of the proteins are nuclear, but it is unclear whether the nuclear and cytoplasmic fractions represent the same number of cell equivalents, or whether the fractions were normalized by total protein amount, which could be misleading. This should be clarified.

Does immunocytochemical staining of these cells with antibodies against p-ErbB proteins yield similar results as the cell fractionation?

Discretionary revisions

Given that EGFR is the only ErbB family member with a clinically validated role in colorectal cancer, it is surprising that EGFR status was not assessed. This would provide valuable context to the current data since multiple ErbB proteins are often co-activated in the same tumor and different combinations of active ErbB receptors can dictate different outcomes. The authors should at least discuss
how they think their data on the prognostic value of p-ErbB3 and p-ErbB4 fits into the context of EGFR signaling in this indication.

**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