Reviewer's report

Title: Impaired degradation followed by enhanced recycling of epidermal growth factor receptor caused by hypo-phosphorylation of tyrosine 1045 in RBE cells

Version: 3 Date: 18 April 2012

Reviewer: Nancy L Lill

Reviewer's report:

The manuscript by Gui et al. reports that impaired EGF receptor degradation in the RBE cholangiocarcinoma cell line correlates with a specific reduction in ligand-induced phosphorylation of EGFR Y1045, the recruitment site for c-Cbl. Phosphorylation of receptor tyrosine 1068 is not compromised, so a gross defect in the kinase activity of the receptor is not to blame. Through complementary protein biochemistry and cell biology approaches, the authors show that the RBE cells have a specific defect in Cbl recruitment, which results in increased receptor recycling, decreased receptor colocalization with the late marker LAMP-1, and prolonged MAP kinase signaling. The report is significant in its identification of another human cancer (cholangiocarcinoma) whose aberrant signaling can be linked to defects in the control of EGFR by Cbl. Further significance derives from the parallel between this work and previously published reports of Y1045 hypophosphorylation/failure to recruit Cbl to EGFR following cell stimulation with the ligand amphiregulin (reports from the Lill, Ethier, Raymond, and van Deurs labs).

The quality of the data is high. The conclusions drawn are appropriate for the results shown. A reasonable next step would be the analysis of patient-derived cholangiocarcinoma tissues, in order to determine the frequency of Cbl/EGFR deregulation in these tumors. In the discussion of the implications of their work, the authors propose that the enhancement of Y1045 phosphorylation could be an important approach to improve anticancer therapeutics. It is not clear now to the reviewer how that might be achieved, but the concept is interesting. Future investigations will be needed to translate the concept to the clinic.

Major compulsory revisions required: none. All of the reviewer's concerns from the initial review have been addressed by text revisions or the inclusion of new
data whose quality is high.

Minor essential revisions: none.

Discretionary revisions suggested: none

**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.