Reviewer's report

Title: Germ-line mutations in Epidermal growth factor receptor (EGFR) are rare but may contribute to oncogenesis: a novel germ-line mutation in EGFR detected in a patient with lung adenocarcinoma

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Reviewer: Daphne Bell

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Centano et al sequenced exons 18-21 of EGFR from 71 lung tumor samples. Somatic EGFR mutations were present in 12 tumors; one of the mutated tumors also had a germline EGFR mutation (p.R776G), which was determined to be in cis with a somatic p.L858R mutation. Rare germline EGFR mutations have previously been associated with familial lung cancer. In the present study, the proband reportedly had a sibling with cancer but no detailed family history could be obtained. The germline R776G variant was not found among a population of 912 lung cancer patients, nor among 477 healthy blood donors indicating that it is rare within the general population. Introduction of constructs expressing wildtype-EGFR of the R776G-EGFR mutant into 293EBNA and COS7 cells, followed by immunoblotting, indicated that the R776G mutant was activating since its expression lead to higher levels of EGFR autophosphorylation that did wildtype EGFR. The authors conclude that germline EGFR mutations are rare but contribute to oncogenesis.

Major Compulsory Revisions:

1. The strategy used to determine whether the p.R776G and p.R858W mutants occurred in cis or in trans was based on restriction digestion followed by sequencing of digested products. The authors report that the R858L mutation was on a 248bp fragment and therefore in cis with the R776G mutation. The concern with the data presented in Figure 1 is that the sequence trace shows the presence of both a wildtype peak and a mutant peak indicating a heterozygous position. If individual digested products were sequenced one would expect to see a wildtype peak or a mutant peak associated with the 248bp fragment, but not both.
2. Figure 1: It is unclear why the schematic diagram of the wildtype (WT) and mutant alleles shows the L858R mutation on both alleles.
3. Figure 2: A loading control should be provided for both Western blots.
4. The authors should determine the biochemical consequences of the p.R776G and p.R858W mutants in cis because, i) this is the configuration in which they are reported to occur in the tumor. Previous studies have shown that in lung tumors, germline and somatic EGFR mutants that occur in cis functionally synergize in vitro (Godin-Heymann et al., Cancer Research 2007;67:7319-26), and ii) in vivo
studies have shown that EGFR(T790M)-expressing mice develop tumors with longer latency than EGFR(L858R+T790M)-bearing animals (Regalkes et al., PLoS One. 2007;2:e810).

Minor Essential Revisions:

5. Two additional references should be cited:

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I am a co-inventor on a patent describing EGFR mutations, which is licensed to Genzyme; I receive royalties resulting from the license.