Author's response to reviews

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

Authors:

Irene Centeno (centeno.ramos@gmail.com)
Pilar Blay (pilarblayalbors@gmail.com)
Iñigo Santamaria (isr@hca.es)
Aurora Astudillo (astudillo@hca.es)
Ana S Pitiot (anapitiot@uniovi.es)
Fernando G Osorio (fgosorio@degradome.uniovi.es)
Patricia Gonzalez-Arriaga (laboepic.uo@uniovi.es)
Fernando Iglesias (fernandoiglesiasl@hotmail.com)
Primitiva Menenedez (tiva@hca.es)
Adonina Tardon (atardon@uniovi.es)
Jose M Freije (jmf@uniovi.es)
Milagros Balbin (mbalbin@hca.es)

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Author’s response to reviews:

Dr Jack Cochrane
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Oviedo, March 25, 2011

Dear Editor:

Please find enclosed a revised version of our manuscript entitled “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” by Centeno et al.

Regarding the reviewer’s reports and concerns, we have addressed all the points as follows:

Major compulsory revisions from reviewer D.B.

1. Concerning the strategy to determine whether p.R776G and p.L858R mutants occurred in cis or in trans, we have repeated the experiment to improve the electrophoretic separation of the fragments corresponding to wild-type and R776G alleles, in order to prevent contaminating wild-type DNA when sequencing the mutant allele. After repeating the complete procedure on the
original tumour sample, we have confirmed that the 248 bp fragment containing
the R776G mutation had a DNA sequence corresponding to homozygous L858R
in cis, as can be seen in the new version of Figure 1. In fact, HaeIII restriction site
is just a few bases away from L858R codon and no more sequence can be read.

2. We have rewritten the diagrams in Fig 1 in order to make them more
understandable.

3. Figure 2. We provide loading controls in Western blots. We have to point out
that these loading controls were made by running new electrophoresis gels with
the same protein extracts since protein bands corresponding to beta-actin were
run off the gels used for the EGFR western blots to achieve an optimal resolution
for high molecular weight proteins.

4. Regarding the request from the reviewer to determine the biochemical
consequences of the p.R776G and p.R858L in cis, we just focused our effort in a
preliminary assay on p.R776G mutation since this is the one appearing as
germline and we wanted to stress in this point. The obtained results indicating
that there is an increase in ligand-independent phosphorylation may suggest an
oncogenic effect for this mutation alone, as has also been suggested in some
studies for the T790M mutation. The fact that a second in-cis mutation is present
within the tumor, in a similar way to the more studied T790M/L858R mutant,
suggests that the oncogenic potential might be enhanced in the double mutant.
We agree that it could be interesting to study more in depth the biochemical
consequences of the double mutation. However, our preliminary results from the
transfection of a construct containing the double mutant are not consistent
enough to provide conclusive information. Consequently, in the present
manuscript we have chosen to present only the very reproducible results
demonstrating the constitutive activation of the novel germline mutation, with the
hope that this information in its present form can be of value. We explain in a
new paragraph within the discussion section about alternative approaches to the
functional analysis of these mutants.

Minor essential revisions from D.B.:

5. We added in the text the two references suggested by the reviewer.

Reviewer E.G.:

1. We have modified figures 1 and 2. We hope that they are now perfectly
understandable and can be correctly read. Figure is on a vector file format (.ppt),
thus retaining its sharpness even when greatly magnified.

2. We added a new paragraph in the discussion regarding alternative analysis
that can be done for studying in vitro the role of those mutations, but we agree
with this reviewer that, taking in account the information already described in this
manuscript, this would not be the main objective of this report.

3. We added the reference suggested by the reviewer.
We state in our methods section that the studied samples were obtained with informed consent given by the patients to participate in research study. The research was approved by the Hospital Ethics Committee.

We would like to thank the reviewers for all their comments and suggestions since they have allowed improving significantly our manuscript and reinforcing the importance of the reported results.

We remain considering that the information provided in this study is of interest and highlights the hypothesis that germ-line mutations in the EGFR gene could be involved in lung cancer pathogenesis.

Thanking in advance for your consideration

Sincerely,

Milagros Balbin, PhD
Laboratorio de Oncologia Molecular
Instituto Universitario de Oncologia del Principado de Asturias-IUOPA
Hospital Universitario Central de Asturias
33006 Oviedo Spain

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