Author's response to reviews

Title: A novel mutation in the tyrosine kinase domain of ERBB2 in hepatocellular carcinoma

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Version: 2 Date: 3 October 2006

Author's response to reviews: see over
Response to Reviewer 1 report

Title: ERBB2, but not EGFR, mutations in hepatocellular carcinoma may predict response to EGFR-targeted therapy

Version: 1 Date: 20 September 2006

Reviewer: Daphne Bell

Reviewer’s report:

General
Bekaii-Saab et al., report a mutational analysis of exons 18-21 of both EGFR and ERBB2 among a series of 18 hepatocellular carcinomas and 22 biliary carcinomas. No mutations were detectable within EGFR in this series. As a positive control for EGFR mutation detection, 2 of 44 NSCLCs were shown to have an EGFR mutation. Mutations affecting ERBB2 were found in two cases of hepatocellular carcinoma. In each case the mutation substituted a tyrosine for histidine at position 878 (H878Y). At least in one tumor, the mutation was shown to be absent within matched control DNA. The authors conclude “mutations in the tyrosine kinase domain of ERBB2 in hepatoma may underlie responsiveness to agents that target ERBB2 and/or EGFR”.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

(1) The conclusion of the text that “mutations in the tyrosine kinase domain of ERBB2 in hepatoma may underlie responsiveness to agents that target ERBB2 and/or EGFR”, as well as the title of the paper “ERRB2, but not EGFR, mutations in hepatocellular carcinoma may predict response to EGFR-targeted therapy”, are over-stated. This study analyzed tumors from unselected (untreated) cases. While it is reasonable to speculate that the single, recurrent ERBB2 mutation identified may be germane to response to EGFR-targeted therapies, it should not be presented so definitively.

- The title was changed to “A novel mutation in the tyrosine kinase domain of ERBB2 in hepatocellular carcinoma”
- The abstract conclusion was changed to the following:” These newly described mutations may play a role in predicting response to EGFR-targeted therapy in hepatoma and their role should be explored in prospective studies.”
- The discussion has been revised to include the following statement “we speculate that these newly described mutations may play a role in predicting response to EGFR-targeted therapy in hepatoma ....”

(2) The abstract states that “11% of hepatomas ... harbored novel mutations in the activating domain” of ERBB2. This is misleading as it implies that multiple novel mutations were found. The abstract should be changed to accurately summarize the findings that a single novel mutation (H878Y) was found within ERBB2, which was recurrent in nature, being observed in two of 18 cases (11%).

This has been changed.
The authors should state in the degree of tumor cellularity (as a %) of the analyzed cases within the materials and methods whether the tumors were reviewed for cellular heterogeneity. Were they enriched for tumor cell content by gross or laser-capture microdissection? This information is critical in indicating whether the findings reported here are likely to be an accurate reflection of the overall incidence of EGFR and ERBB2 mutations in these tumor types, or whether they are likely an underestimate of the true mutation frequency.

The hepatobiliary cancers were laser capture microdissected to enrich the neoplastic component. This has been stated in the Methods section.

The authors have not provided the nucleotide change that leads to the H878Y missense mutation within ERRB2. Was it the same nucleotide substitution in each case? This information should be added to the results as well as the figure legend. The protein change should be stated in the abstract.

The nucleotide change is CAT to TAT (c.2632 C>T). This mutation is illustrated in Figure 1 and the exact nucleotide mutation stated in the Results. Both cases have the same nucleotide substitution and this has been clarified in the Results.

Were the two mutation-positive cases of hepatocellular carcinoma different from the mutation-negative cases in terms of their associated clinicopathological features?

All cases were confirmed hepatomas. Given the relative small numbers (2 vs 16), it is difficult to say if the two with mutations are different than the remaining 16 with respect to clinico-pathologic features.

Was matched normal tissue genotyped for both cases? While this is implied in the abstract, it should be stated implicitly with both the Materials and Methods and the Results.

The following statement was added to Materials and Methods: “All samples were tested against matched normal (germline) DNA”

The following statement was added to the results: “None of the matched normal tissue was found to have the somatic mutations for EGFR or ERBB2 found in the tumors.”

The methodology provided is insufficient in detail to permit replication of the work. The authors should provide details of the primers and PCR conditions used for mutational analysis of ERBB2 in this study.

The ERBB2 primers are standard and have been directly adapted from the original Stephens et al. Nature 2004 paper. This has been stated in the Methods section.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

(8) The authors should explicitly state that the mutational analysis of EGFR in a series of NSCLCs was conducted as a positive control for mutation detection, rather than simply a positive control.

These were added.

(9) The conclusion should be combined with the discussion

This was done.

Discretionary Revisions (which the author can choose to ignore)

(10) Can the authors provide data on the genotype of ERBB2 exons 18-21 in an expanded series of primary hepatocellular carcinomas or cell-lines derived from these tumors?

We apologize that we are unable to provide expanded mutational data for a larger series of these extremely rare solid tumors. See below.

(11) Do the authors have access to any cases of hepatocellular carcinoma that showed a response to EGFR-ERBB2-targeted therapies, for mutational analysis?

We are only now gearing up to begin a clinical trial (phase I-II) where eventually we would be able to obtain specimens with response data.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No
Declaration of competing interests: I am an inventor on a patent application describing EGFR mutations and receive royalties resulting from the licensing of a genetic test for EGFR mutations.

Response to Reviewer 2 report

Title: ERBB2, but not EGFR, mutations in hepatocellular carcinoma may predict response to EGFR-targeted therapy
Version: 1
Date: 20 September 2006
Reviewer: Balazs Halmos
Reviewer's report:

General

Bekaii-Saab et al report the results of a study looking at EGFR and ErbB2 mutations in tumor specimens from patients with hepatocellular cancer and biliary cancers. They claim the identification of novel ErbB2 mutations in 2/18 hepatocellular cancer specimens and conclude that these new mutations might predict responsiveness to EGFR-targeted therapy in hepatoma. While their study goals are relevant and the results could potentially be interesting, there are a number of issues, such as the limited scope of the study, lack of in vitro confirmatory studies and lack of correlation with clinical response that limit enthusiasm for the ready acceptance of their conclusions.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Their title, abstract and conclusions all state that the ErbB2 mutations they identified “may predict”, “may be germane in predicting”, “may underlie responsiveness” to EGFR tyrosine kinase inhibitor therapy. I believe such statements are unfounded and misleading- they do not provide any information, either from clinical data or in vitro testing that would suggest that this is indeed true. In fact, ErbB2 mutations identified in non-small cell lung cancers are felt to be relatively refractory to EGFR TKIs. I believe these statements should be appropriately restated as speculation and in particular the title should be changed.

- The title was changed to “A novel mutation in the tyrosine kinase domain of ERBB2 in hepatocellular carcinoma”
- The abstract conclusion was changed to the following:” These newly described mutations may play a role in predicting response to EGFR-targeted therapy in hepatoma and their role should be explored in prospective studies.”
- The discussion now includes the following statement “we speculate that these newly described mutations may play a role in predicting response to EGFR-targeted therapy in hepatoma ….”

2. The methods section should be described in more detail. Were the specimens paraffin-embedded? How was DNA extracted? Were the tumors microdissected? What were the normal controls that they used? Normal liver for all (that seems to be the case at least for one of the mutants as shown in the figure)? PBMCs? Were the normal specimens processed the same way as tumor? E.g. Paraffin-embedding can lead to PCR artifacts due to deamination of DNA. They do not list what primers they used for ErbB2 sequencing. They state they did double-stranded sequencing for EGFR- did they do the same for ErbB2? If so, was the H878Y mutation confirmed in both directions?

The lung adenocarcinomas were frozen samples while all hepatobiliary (and their matched normal tissues) were paraffin-embedded. All sequencing is standard double stranded sequencing in both directions. All amplicons with mutations or variants were sequenced in both directions in addition to having
a new independent amplicon derived and re-sequenced in both directions. The Eng lab is very experienced in using even very small amounts of template from paraffin-embedded tissue as illustrated by utilization of tumor stromal cells for multiplex-PCR-based whole genome scanning with minimal artifact (see Kurose K et al. HMG 2001, Nature Genet 2002, Fukino K et al. Cancer Res 2004). The amounts of tissue used in this study were generous compared to the stromal studies. Further, the fact that two independent amplicons result in the same mutation, and corresponding germline (from paraffin-embedded tissue too) do not show any mutation confirms that these mutations are not artifactual.

3. The identification of such a novel mutation would certainly be of interest but the validity of their findings should also be further strengthened by subcloning the DNA and/or confirming the same mutation in RNA to mitigate the concern that it might just represent an artifact due to paraffin-embedding (assuming the specimens were paraffin-embedded). They state that they used 44 lung cancers as controls but do not describe the ErbB2 sequencing results on those samples- were ErbB2 mutations identified in those?

We are sorry that we cannot obtain RNA from these relatively small amounts of hepatobiliary tumors. As noted above, we believe that the two independent amplifications yielding identical results with no variants noted in the equivalent germlines (also derived from paraffin tissue) give strong evidence that these two detected mutations are not artifactual.

Discretionary Revisions (which the author can choose to ignore)

1. The H878Y mutation they report in 2 hepatoma specimens are hypothesized to affect function but no attempt is made to confirm this in in vitro assays. Given the fact that these would represent novel mutations not described before, the functional consequences cannot be well predicted at this point without such studies and their conclusions would be greatly strengthened by such.

Of course, functional characterization is nice, but unfortunately, we are not a lab which assays for function in this molecule.

2. No clinical information is provided on the 2 patients whose tumor contained an ErbB2 mutation- it would be interesting to learn if there were any particular clinical or pathological factors that might correlate with the presence of these mutations (e.g. gender, history of hepatitis/alcoholism/estrogen use etc...). If such clinical data is available, it should be provided.

Given the relative small numbers (2 vs. 16), it is difficult to say if the two with mutations are different than the remaining 16 with respect to clinico-pathologic features.
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No
Declaration of competing interests: I declare that I have no competing interests