Author's response to reviews

Title: Mutations in epidermal growth factor receptor and K-ras in Chinese patients with colorectal cancer

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Author's response to reviews: see over
Dear Editor:
Thanks to your reviewers for their constructive comments. The comments have helped us to make the manuscript more scientific and clear. We have made some changes to the manuscript after carefully checking the data. The answers to the questions and explanations for the changes are listed below. Thank you very much for your time and further consideration.

Sincerely yours,

Jun Cao

**Question by Alberto Bardelli:**
**Q: the sentence on page 13 is unclear:**
EGFR mutations are rare in Chinese patients with CRC, which is to the results for Chinese patients with NSCLC.

**Answer:** The word “similar” should be added before “to the results for Chinese patients with NSCLC”.
EGFR mutations are rare in Chinese patients with CRC, which is similar to the results for Chinese patients with NSCLC.

**Questions by fahd al-mulla:**
**Q1. The authors need to clarify the selection process of patients. Do these patients from Fudan Hospital represent the Chinese population? How were they selected?**
*There seem to be a bias towards stage IV cases, which accounted for 24.8% of cases. This needs to be clarified.*

**Answer:** The 101 tumor samples were all obtained from operations performed at the Fudan University Cancer Hospital between January 2004 and March 2006, that had complete medical information. Because Fudan University Cancer Hospital is one of the best specialist cancer hospitals in China and 60% of the patients come from other
areas of China, the sample is representative of the whole Chinese population. According to the NCCN guideline, patients with presumably resectable liver metastasis (stage IV) can receive radical operations. Many such patients from other provinces who are thought by local doctors to have unresectable disease attend this hospital for radical surgery. This explains why a large proportion of stage IV cases were included in our study.

**Q2. The authors stated that 8-10µm sections were carefully dissected. What does this mean? Were the sections microdissected?**

**Answer:** The slides were first selected under the microscope to ensure that they contained sufficient tumor material. The paraffin-embedded tumor tissue blocks were then dissected into 8-10µm sections for PCR sample preparation.

**Q3. The cycling conditions of the PCR need to be adjusted for EGFR and Ki-ras. Were they both the same? For external and internal primers?**

**Answer:** The cycling conditions of the PCR were different for EGFR and Ki-ras. We have corrected this in the article. We used the same cycling conditions for external and internal primers.

**Q4. The authors do not account or comment much on the lack of codon 61 of Ki-ras gene in their population.**

**Answer:** It has been reported in Western studies that approximately 90% of the activating mutations were found in codons 12 and 13 of exon 1 and 0~5% in codon 61 located in exon 2 (Kisliitsin D et al. 2002; Breivik J et al. 1994; Vogelstein B et al. 1988). Similarly, the mutation incidence of codon 61 was 0~4.8% in domestic reports. In this study, no mutations in codon 61 were found, which is concordant with that of previous studies.

**Q5. The authors did not expand much on the novel mutations they discovered. Are the mutations in codons 45, 69 and 80 synonymous or unsynonymous mutations? The authors made bold statements about these novel mutations in the discussion regarding biological behavior and drug resistance, yet they did not expand on their translational relevance?**
The mutations in codons 45, 69 and 80 were all unsynonymous mutations, as shown in “Table 2”. We haven’t yet expanded on translational relevance because of the low incidence of the 3 mutations found. Only one of each mutation in codons 45, 69 and 80 was found in this study. That’s why no statements about these novel mutations regarding biological behavior and drug resistance were made in the discussion.

**Q6. The discussion magnified the role of antibody therapy. However, we were not informed how these patients were treated?**

**Answer:** The patients in this study were mostly treated between 2004 and 2007. Cetuximab was unavailable until July, 2006 and panitumumab is still unavailable at present in China. Furthermore, some of the patients were not given cetuximab when they experienced recurrent or metastatic disease for financial reasons. Only 13 patients in this study received cetuximab as first- to third-line treatment. As the data on cetuximab treatment are limited, we discussed the anti-EGFR antibody and relationship between K-ras mutation and cetuximab efficacy.

**7. The manuscript needs serious English editing. There are numerous grammatical and spelling errors that need to be addressed.**

**Answer:** We have asked a native English speaker to revise the manuscript and make the necessary corrections.