Format for ANSWERING REVIEWERS

Oct 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12624-revised.doc).

Title: KRAS, BRAF gene mutations and DNA mismatch repair status in Chinese colorectal carcinoma patients

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer.

(1) Codon 12 and 13 of RAS gene is within exon 1. How exon 2 screening gave information on these two mutations.
According to the standard nomenclature recommendations of the HGVS (http://www.HGVS.org/mutnomen/), "Coding DNA reference sequence" refers to a cDNA derived sequence containing the full length of all coding regions and non-coding untranslated regions (5'-UTR and 3'-UTR). In the NCBI reference sequence of KRAS (GenBank accession number.: NM_033360.3), the exon 1 belongs to 5' untranslated region and the start codon is located on the exon 2. Thus, gene screening of KRAS exon 2 included the mutations on codon 12 and 13.

(2) What is the status of codon 61 of RAS gene?
It was not until late 2014 did the NCCN guideline for colorectal cancer recommend that non-exon 2 KRAS mutation status should also be determined. According to Tong et al, the frequencies of KRAS mutations at its codons 61 and 146 are 2.5% and 2.7% respectively in Chinese sporadic colorectal cancer patients (Cancer Biol Ther. 2014;15:768-76). Now, we have included the codons 61 and 146 in our clinical molecular test for colorectal cancer. But the data were not enough to integrate to this paper.

(3) It would be appropriate to provide few representative chromatograms showing mutations. More importantly, for rare ones.
The representative chromatograms showing concomitant mutations of KRAS and BRAF have been included in the revised manuscript as Figure 1.

(4) Why ARMS PCR was done for few tumor samples?
In our clinical practice, ARMS PCR was only used in small biopsy specimens with less tumor cells and more infiltrating lymphocytes. However, most patients with colon cancer would accept tumor resection after biopsy, therefore in most of the cases, Sanger sequencing for mutation analysis were performed.

(5) Representative IHC photograph should be shown.
Representative IHC photograph maybe helpful in understanding the staining pattern of MMR proteins, however, the aim of our study is to declare the frequency of KRAS, BRAF mutations and MMR deficiency in Chinese CRC patients. A non-otherwise specific IHC photograph does not appear to be helpful in better understanding the core aim of our study. Nevertheless, we attached an IHC photograph in the revised manuscript as a supplementary figure in case it is needed.

3 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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