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Format for ANSWERING REVIEWERS

May 23, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10664-review.doc).

Title: Common TERT promoter mutations in hepatocellular carcinomas from different geographical locations

Author: Dilek Cevik, Gokhan Yildiz, Mehmet Ozturk

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 10664

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated and has been edited for language by a English language expert.

2 Revision has been made according to the suggestions of the reviewers

(1) First reviewer: 1) please specify whether these patients carry germline mutations: **The mutations that we described in the paper are most probably somatic, since all previously reported TERT promoter mutations in HCC were somatic, but not germ-line. We added this information in the manuscript section Results-Tert Promoter Mutations in Primary Hepatocellular Carcinoma Tumors, page 8**. 2) add a figure with the geographic distribution and different prevalence of the TERN mutations: **We added a new figure (Figure 2) as requested.** 3) which was the primary cause of the selected tumors (NAFLD, HCV,HBV, etc)?: **Tumors studied here are from an archive that we have been studying since 1991. There has been no selection criteria applied for this study. We were able to trace the HBV status in these tumors based on our previous papers. Accordingly, we updated this HBV-related information in tables 2 and 3. In total, 23 out 44 tumors reported here were positive for HBV DNA, whereas 18 were HBV DNA negative.**

(2) Second reviewer: Although it is an interesting study, number of patients from Africa is fewer. So patients from Africa ought to be added to 20 cases. **As explained, we used archived HCC samples for this study and have no access to novel tumors from Africa. Therefore, the number of African samples remained as 15.**

(3) 1- In the methods section : what is the density of cells seeded in the culture medium.Is the starting number of cells seeded affects the genomic DNA concentrations isolated by using Purelink Genomic DNA Kit. **The cell density was 70% before DNA extraction. This information was added in Materials and Methods section-page 6. We did not check whether star**ting number of cells affected DNA recovery using Purelink Genomic DNA Kit. 2- In discussion the authors reported that TERT promoter mutations have been reported recently as a frequent event in some cancers including HCC. Why the author selected HCC specifically for this study. **The reasons for choosing HCC for further TERT promoter studies were provided in the Introduction Section-page 5. In short, there was no mutation data for HCC tumors originating from Asia and Africa in two published papers.** 3- In discussion the authors reported that somatic mutations of TERT promoter are among the most frequent aberrations observed in some tumor types. Please indicate these tumor types. **Tumor types with high frequency of TERT mutations were named in Discussion section-page 9.** 4- In discussion : Cancer cells including HCC cells overcome this arrest by reactivating TERT gene expression with ill-known mechanisms. What is ill-known mechanisms (clarify). **We modified the related text in Discussion section-page 9 to include known mechanisms of TERT activation in cancer cells.** 5- In results section the authors stated that Both epithelial-like and mesenchymal-like cells had these mutations with similar frequencies. Please give a more detailed discussion about this finding. **We added our comments on this matter in Discussion section page 9**. 6- In results section (TERT promoter mutations in primary hepatocellular carcinoma tumors) the authors used archival collection of 44 hepatocellular carcinoma tumor DNAs collected from different countries around the world including Japan (11 patients), China (8), Germany (7), France (2), Israel (1), Mozambique (6), Transkei (4), Lesotho (2), Swaziland (1) and South Africa (2). Why the author did not use the same number of patients from different countries. How can I judge the results (regarding geographical location African to non-African) from sample of 11 (Japan) to sample of one (Swaziland) and South Africa (2). **As explained before, we used archived tumor DNA samples for these studies. We do not have a larger archive to make a selection. All available DNA samples were included in this study. We provided geographical data for tumors reported here for descriptive reasons. This is why we did not attempt to compare tumors from different countries. Instead we compared African versus Non-African tumors.**

(4) Some sample size is not large enough to have reach clear-out conclusion. **As explained before, we used archived tumor DNA samples for these studies. We do not have a larger archive to make a selection. All available DNA samples were included in this study. We provided geographical data for tumors reported here for descriptive reasons. This is why we did not attempt to compare tumors from different countries. Instead we compared African versus Non-African tumors.**

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References and typesetting were corrected and whole text has been edited for English by a specialist.

We believe that one should use "**Observational Study**" indication for the Columns Scope.

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

Sincerely yours,

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