ANSWERING REVIEWERS

April 26, 2016

Dear science Editor Jing Yu and reviewers,

Thank you for your positive comments and detailed instructions for further revision of our manuscript "Relationships between cell cycle pathway genes polymorphisms and the risk of hepatocellular carcinoma". I have done the requested revisions and highlighted them in red.

Please find enclosed the edited manuscript in Word format (file name: 25314-Revised manuscript).

Name of journal: World Journal of Gastroenterology

Manuscript NO.: 25314

Title: Relationships between cell cycle pathway genes polymorphisms and the risk of hepatocellular carcinoma

Authors: Yue-Li Nan, Yan-Ling Hu, Zhi-Ke Liu, Fang-Fang Duan, Yang Xu, Shu Li, Ting Li, Da-Fang Chen and Xiao-Yun Zeng

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

Responses to Reviewer 1 (Reviewer's code: 03478148**)**

"The manuscript is interesting and providing relevant information. The study is well planned and including large no. of patients as well as control. The manuscript contains many typographical and grammatical errors which are need to be corrected."

Reply: Thank you for your kind reviewing and advice sincerely. I'm sorry for the typographical and grammatical errors. I've corrected the sentences which are not well expressed. Furthermore, the whole manuscript has already undergone a comprehensive English revision process by an editing company "Edanz". The certificate is attached.

Responses to Reviewer 2 (Reviewer's code: 02542093**)**

"Comments:? The authors have to justify why those 15 SNPs were chosen to assess the association with HCC.? It would be interested if the grade of HCC was associated with those SNPs."

1. ? The authors have to justify why those 15 SNPs were chosen to assess the association with HCC.?

Reply: Thank you for your kind reviewing and advice sincerely. The reason that those 15 SNPs were chosen to assess the association with HCC as follows:

SNP selection

From the GEO database (https://www.ncbi.nlm.nih.gov/geo/), we found three sets of whole genome expression microarray data which were related to HCC (GSE14520, GSE25097, GSE12941). 3826 different genes were selected using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) (p<0.05). Gene ontology classification and pathway enrichment analysis was performed by blast2GO and DAVID (https://david.ncifcrf.gov/) and 40 cell cycle pathway genes involved in the cellular process were chose. Download the genotype data from Hapmap (http://hapmap.ncbi.nlm.nih.gov), then performed Haploview 4.2 (Cambridge, MA o2141, USA) and function prediction website (http://snpinfo.niehs.nih.gov/snpfunc.htm) to selected functional SNPs (MAF>0.05, r^2>0.8). Through the reference to existing literature on these SNPs with HCC, 15 SNPs form 12 genes (*MCM4* rs2305952，*YWHAB* rs2425675，*CDKN2A* rs3088440，*TGFB3* rs3917148，*RBL2* rs3929，*RAD21* rs6987652，*SMAD3* rs11556090, rs8025774，*KAT2B* rs17006625, rs4858770，*MCM7* rs2070215, rs2261360，*CDKN1A* rs3176320，*CDC25C* rs3734166，and *CHEK1* rs515255) were selected in this study. Information of selected SNPs is shown in table 1.

Table 1

The condition of functional SNPs from cell cycle pathway genes

Genes	SNPs	Chromosome (position)	Allele	MAF （hapmap-HCB）
MCM4	rs2305952	8 (47962049)	C/T	C=0.18
YWHAB	rs2425675	20 (44906293)	A/G	A=0.20
CDKN2A	rs3088440	9 (21968160)	A/G	A=0.08

TGFB3	rs3917148	14 (75980178)	A/C	C=0.10
RBL2	rs3929	16 (53490396)	C/G	C=0.20
RAD21	rs6987652	8 (116870042)	A/G	A=0.12
SMAD3	rs11556090	15 (67194045)	A/G	G=0.09
	rs8025774	15 (67190938)	C/T	C=0.45
KAT2B	rs17006625	3 (20119604)	A/G	G=0.14
	rs4858770	3(20152931)	C/T	T=0.47
MCM7	rs2070215	7 (100099174)	A/G	G=0.29
	rs2261360	7 (100095370)	A/C	A=0.37
CDKN1A	rs3176320	6 (36679011)	A/G	G=0.17
CDC25C	rs3734166	5 (138329634)	A/G	G=0.38
CHEK1	rs515255	11 (125627250)	C/T	T=0.44

2. It would be interested if the grade of HCC was associated with those SNPs.

Reply: Thank you for your kind reviewing and advice sincerely. We agree with you that it is may be more interesting if the grade of HCC was associated with those SNPs. In this study, all patients were recruited from June 2007 to September 2013 in the Tumor Hospital of Guangxi Medical University. Therefore, for most subjects, we cannot obtain the data of the grade of HCC.

Responses to Reviewer 3 (Reviewer's code: 03062399**)**

"The paper entitled: Relationships between cell pathway gene polymorphisms and the risk of hepatocellular carcinoma by Nan et al. is a descriptive paper analyzing the polymorphism in HCC in a wide number of patients. The paper is a mere analysis of the SNPs of MCM4, CHEK1 and KATB2. The analysis is well conducted, but no a minimal hypothesis about the mechanism is proposed or experiments are performed. However, the analyses are consistent with the results and the conclusions asserted in the manuscript. The English has to be reviewed to well explain some parts of the paper. The abstract has to be rewrite, it doesn't well synthesise the aim and results of the manuscript."

1. The paper is a mere analysis of the SNPs of MCM4, CHEK1 and KATB2. The analysis is well conducted, but no a minimal hypothesis about the mechanism is proposed or experiments are performed.

Reply: Thank you for your kind reviewing and advice sincerely. In our study, we investigated the association between the polymorphisms of fifteen functional SNPs in twelve cell cycle pathway genes and the risk of HCC and we found only three SNPs of fifteen SNPs (*MCM4* rs2305952, *CHEK1* rs515255, and *KAT2B* rs17006625) were significantly associated with the risk of HCC. We have added the mechanism of three significant genes (*MCM4*, *CHEK1* and *KAT2B*) by referring other researches. The functional influence of the examined SNPs and the potential mechanisms there of need to be determined in functional validation tests and the experiment are now underway.

2. The abstract has to be rewrite, it doesn't well synthesise the aim and results of the manuscript.

Reply: Thank you for your kind reviewing and advice sincerely. The abstract has been rewrite as follows:

Abstract

AIM: To investigate associations between the polymorphisms of cell cycle pathway genes and the risk of hepatocellular carcinoma (HCC).

METHODS: We enrolled 1127 cases newly diagnosed with HCC from the Tumor Hospital of Guangxi Medical University and 1200 non-tumor patients from the First Affiliated Hospital of Guangxi Medical University. General demographic characteristics, behavioral information, and hematological indices were collected by unified questionnaires. Genomic DNA was isolated from peripheral venous blood using Phenol-Chloroform. The genotyping was performed using the Sequenom MassARRAY iPLEX genotyping method. The association between genetic polymorphisms and risk of HCC was shown by *P* value and the odd ratio (OR) with 95% confidence interval (CI) using the unconditional logistic regression after adjusting for age, sex, nationality, smoking, drinking, family history of HCC, and HBV infection. Moreover,

stratified analysis was conducted on the basis of the status of HBV infection, smoking, drinking alcohol.

RESULTS: The HCC risk was lower in patients with the *MCM4* rs2305952 CC (OR=0.22, 95%CI: 0.08–0.63, *P*=0.01) and with the *CHEK1* rs515255 TC, TT, TC/TT (OR=0.73, 95%CI: 0.56–0.96, *P*=0.02; OR=0.67, 95%CI: 0.46–0.97, *P*=0.04; OR=0.72, 95%CI: 0.56–0.92, *P*=0.01, respectively). Conversely, the HCC risk was higher in patients with the *KAT2B* rs17006625 GG (OR=1.64, 95%CI: 1.01–2.64, *P*=0.04). In addition, the risk were markedly lower for those who were carriers *MCM4* rs2305952 CC and were also HBsAg-positive and non-drinking and non-smoking (*P*<0.05, respectively) and for those who were carriers *CHEK1* rs515255 TC, TT, TC/TT and were also HBsAg-negative and non-drinking (*P*<0.05, respectively). Moreover, the risk were higher for those who were carriers *KAT2B* rs17006625 GG and were also HBsAg-negative (*P*<0.05).

CONCLUSION: Of twelve cell cycle pathway genes, *MCM4*, *CHEK1* and *KAT2B* polymorphisms may be associated with the risk of HCC.

Key words: Cell cycle pathway genes; Single nucleotide polymorphism; Hepatocellular carcinoma; Genetic susceptibility; Case-control study

I sincerely hope that you consider our manuscript eligible for publication in the *World Journal of Gastroenterology*.

Best regards,

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