February 6, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8465-review.doc).

Title: Tumour suppressor HLJ1: a potential diagnostic, preventive and therapeutic target in non-small cell lung cancer

Author: Meng-Feng Tsai, Chi-Chung Wang, Jeremy J.W. Chen

Name of Journal: *World Journal of Clinical Oncology*

ESPS Manuscript NO: 8465

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

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the "*World Journal of Clinical Oncology*".

Sincerely yours,

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Response to Reviewer 1 ESPS Manuscript NO: 8465

Comments from Reviewer:
Dr.chen et al. review heat shock protein HLJ1 as a potential diagnostic, preventive and therapeutic target in non-small cell lung cancer. This is a very clearly-written and exceptionally well-structured manuscript that discusses a timely topic.
There are a few points that need further clarification.

1. Transcriptional regulation of HLJ1. Any evidence to demonstrate the YY1 expression and function regulating HLJ1 in NSCLC.

Response from Authors:
YY1 has a fundamental role in biological and physiological processes such as embryogenesis, differentiation, DNA replication, and cellular proliferation. As a transcription factor, YY1 regulates the expression of numerous genes that are mostly involved in tumourigensis. YY1 overexpression has been demonstrated in several human cancers such as breast cancer, prostate cancer, cervical cancer, brain cancer and colon cancer. In prostate cancer, elevated YY1 expression correlates with higher morphologic grades or malignant histological phenotypes. Similarly, YY1 overexpression in osteosarcoma is positively correlated with the degrees of malignancy. However, functional and clinical analysis of the YY1 in NSCLC remains unclear. We have recently shown that the HLJ1 promoter contain four YY1-binding sites. Overexpression of YY1 in NSCLC cells indicated that up-regulates the tumour suppressor HLJ1 by directly binding to the promoter region, thus inhibiting cancer cell invasion. To address the reviewer's concern about the YY1 expression and function regulating HLJ1 in NSCLC. The related descriptions are added to the section of "Transcriptional regulation of HLJ1" on page 10 of the revised manuscript.

"YY1 can either activate or repress the target genes, depending on the cofactors that it recruits."

"YY1 overexpression has been demonstrated in several human cancers such as breast cancer, prostate cancer, cervical cancer, brain cancer and colon cancer. However, functional and clinical analysis of the YY1 in NSCLC remains unclear."

"As a transcription factor, YY1 regulates the expression of numerous genes that are mostly involved in tumourigensis."

"Overexpression of YY1 in NSCLC cells indicated that up-regulates the HLJ1 expression by directly binding to the promoter region, thus inhibiting cancer cell invasion."

2. HBV protein expression in NSCLC?

Response from Authors:
To our knowledge, there are no any reports to suggest the HBV protein expressed and

involved in lung cancer development. We have rewritten the paragraph to make it clear and concise. The related descriptions are added to the section of “Transcriptional regulation of HLJ1” on page 11 of the revised manuscript.

“Hepatitis B virus (HBV) is a major cause of human hepatocellular carcinoma (HCC). HBV proteins promote migration-related factors such as MT1-MMP, MMP-9, and HIF1A and contribute to the HCC metastatic process. However, HBV could also promote HLJ1 expression in HCC cells by up-regulating the transcription factor YY1. However, the role of the HLJ1 in the metastasis of liver cancer is still unclear.”

3. Page 8, in vovo---in vivo; Page 17, P53,P21 and Figure legend P21----p53,p21

Response from Authors:
All of these typos have already been corrected.

On page 7 of the revised manuscript:
“Tid1-L overexpression in lung cancer cell attenuates EGFR signalling and inhibits cell proliferation, colony formation, and in vivo tumour growth.”

On page 9 and 13 of the revised manuscript:
“The same study also suggested that HLJ1 can affect the expression of many genes downstream in the STAT1 pathway, including p21WAF1, ISGF3G, IFIT1, IFITM1, OAS3, and GIP2 and that HLJ1 can increase p21WAF1 expression by affecting a p53-independent pathway.”

“HLJ1 can inhibit cell cycle progression by increasing the STAT1 and p21WAF1 pathways and by decreasing cyclin D1 expression. The activation of the STAT1 pathway by HLJ1 was independent of p53.”

On page 23 (Figure legend) of the revised manuscript:
“HLJ1 inhibited lung cancer cell proliferation, anchorage-independent growth, cell motility, invasion and tumourigenesis through STAT1/p21WAF1 pathway, HLJ1/NPM1/AP2-α complex, SULG/E-cadherin pathway and modulated cancer -related genes.”

Response to Reviewer 2 ESPS Manuscript NO: 8465

Thank you for your valuable comments. We agreed with all of your comments. Our responses to each of your comments are as follows:

Comments from Reviewer:

This paper is a review paper discussing the biology of the small human heat shock protein HLJ1 (a HSP40 family member) and the current understanding of the molecular mechanisms responsible for the tumor suppressor effects of HLJ1 in lung cancer. This paper further describes endogenous expression regulation mechanisms of HLJ1, potential interactions with herbal medicine (curcumin), and DMSO-dependent regulations of HLJ1expression in relation to these chemical-dependent tumor growth regulations. A majority of the descriptions above are derived mainly from the authors' own extensive research. The authors concluded that HLJ1 can be a realistic biomarker and a potential target for chemoprevention and/or chemotherapy for non-small cell lung cancer. The review subject is important and potentially valuable. It is written in acceptable English, subheadings are organized well, and the story is rationally constructed. If the authors can address the following specific concerns, the quality of the presented paper will be improved further.

1. The most reasonable concern is how the HLJ1 can be a good marker for the early detection of cancer, since the authors have stated that expression of this protein is ubiquitous in many organs and tissues and relatively abundant. The authors' previous study indicates HLJ1 expression in lung cancer tissue is higher than in noncancerous tissue, however, this increase can be easily covered by other tissue damages or abnormal expression in other tissues other than cancer tissue. More rigorous discussion in this issue would be helpful.

Response from Authors:
HLJ1 has not been suggested to be associated with early detection of lung cancer. We correct the mistake and rewrite the sentence to make it clear.

On page 8 of the revised manuscript: "The results of the clinical analyses suggested that HLJ1 is a significant, independent prognostic predictor of recurrence and overall survival in NSCLC patients."

2. The authors' writing pattern gives a description of the scientific discoveries in the beginning of paragraphs without specific citations throughout manuscript. They refrain from citing related papers till the end of the paragraphs. This method is not reader friendly and citations should be more punctual.

Response from Authors:
The specific citations have been rearranged and corrected carefully.

3. Page 13, line 12, the following sentence needs revision since it seems confusing “The effect of deleting an individual binding site on the HLJ1 promoter activation site was differential and limited, with a decrease in activity from 17 to 34%.” Page 14, 5th line from the bottom, the sentence start with “These studies------”: What is the word “these” indicating? Page 16, 2nd line, “curcumin from ginger”: this statement could potentially mislead the reader. It is better to use “from a spice turmeric”.

Response from Authors:

We have corrected the mistakes and rewritten the sentences to make it clear.

On page 10 of the revised manuscript:
“When compared with the WT construct, the mutants of these four potential YY1-binding sites resulted in different levels of reduction in HLJ1 promoter activity, from 17 to 34%, respectively.”

On page 11 of the revised manuscript:
“Curcumin may have potential as a multi-target drug in anticancer therapy.”

On page 12 of the revised manuscript:
“These compounds, including curcumin from a spice turmeric, epigallocatechin-3-gallate from green tea and lycopene from tomato, could target important mechanisms in tumour growth and metastasis.”

Response to Reviewer 3 ESPS Manuscript NO: 8465

Comments from Reviewer:

Thanks for the opportunity to review this interested manuscript entitled: "Tumour suppressor HLJ1: a potential diagnostic, preventive and therapeutic target in non-small cell lung cancer". The manuscript is nicely written. The language is clear. The authors performed a review to suggest that HLJ1 is a potential biomarker and treatment target for NSCLC . They found that drug targeting HLJ1 may be an effective approach for lung cancer therapy by review published papers about HLJ1 structure, function and possible mechanism in LC.I have a few comments for the authors: some languages need to refine.

1. “Human HSP40 and cancer” part, in this article, author main discuss HLJ1 protein, although the HELJ1 was a member of HSP40 family, it was not necessary to describe HSP40 in detail.

Response from Authors:
Thank you for your highly experienced and constructive comments. We have rewritten the section of “Human HSP40 and cancer” to make it clear and concise. The related descriptions are presented on page 6 to 7 of the revised manuscript.

2. In sentence of “The results of Kaplan-Meier survival curve and log-rank analyses suggested that patients with high HLJ1 expression had significantly longer overall survival and disease-free survival times compared to those with low HLJ1 expression.”, “ Kaplan-Meier survival curve and log-rank analyses ” is not meaning.

Response from Authors:
We have rewritten the sentence.

On page 8 of the revised manuscript:
“NSCLC patients with high HLJ1 expression had significantly longer overall survival and disease-free survival times compared to those with low HLJ1 expression.”