Author’s response to reviews

Title: Down-regulation of the tumour suppressor κ-opioid receptor predicts poor prognosis in hepatocellular carcinoma patients

Authors:

Dongtai Chen (chendt@sysucc.org.cn)
Yonghua Chen (chenyh@sysucc.org.cn)
Yan Yan (670589857@qq.com)
Jiaha Pan (panjiah@sysucc.org.cn)
Wei Xing (xingwei@sysucc.org.cn)
Qiang Li (liqiang@sysucc.org.cn)
Weian Zeng (zengwa@mail.sysu.edu.cn)

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Author’s response to reviews:

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Saeed Bajestani, M.D.
Handing Editor, BMC Cancer

Dear Mr Bajestani,

I am very glad to hear from you and thank you very much for your attention and the reviewers’ evaluation and comments on our manuscript, titled “Down-regulation of the tumour suppressor κ-opioid receptor predicts poor prognosis in hepatocellular carcinoma patients”. We have revised the manuscript according to your kind advices and reviewers’ detailed suggestions. All changes and answers to the comments of you and reviewers are listed below.

Once again, thank you very much for the time and patient that you and all reviewers spent on this manuscript. I look forward to hearing from you.
Response to reviewers’ comments

Comments of Ya-Wen Wang (Reviewer 1):

Chen et al found that KOR expression was down-regulated in HCC and KOR was identified as an independent risk factor for both overall survival and recurrence-free survival. The study is interesting and of some novelty.

1. The reason to choose KOR for investigation should be clearly described. Why not other opioid receptors? Is the phenomenon of down-regulation specific for KOR? How about other opioid receptors in HCC?

2. "We graded the samples according to the staining intensity and percentage of cells stained with a score of 0-3". The evaluation methods should be described in more detail.

3. The clinicopathological parameters 64 cases of fresh tumour tissues should also be collected and used for analysis.

4. ROC curves should be constructed.

5. The potential function and mechanism of KOR in HCC should be discussed.

6. It is suggested that Fig 1C and 2A should be presented with higher magnification.

Answer:

Thank you for your suggestions.

1. The reason to choose KOR for investigation should be clearly described. Why not other opioid receptors? Is the phenomenon of down-regulation specific for KOR? How about other opioid receptors in HCC?

Studies of MOR expression and cancer have received the lion's share of attention in recent years. Lu and his colleagues (Lu J. Oncol Rep, 2013, 30(4): 1731-1738) have demonstrated that downregulation of MOR is able to inhibit both in vivo and in vitro human liver cancer progress.
In addition, Tang and his colleagues (Tang B. Int J Oncol, 2013, 43(4): 1281-1290.) have shown that DOR is highly expressed in HCC and upregulation of DOR promotes cancer progression both in vivo and in vitro experiments. However, the expression profile and potential role of KOR in HCC remains unknown. Therefore, we choose KOR for investigation.

2. "We graded the samples according to the staining intensity and percentage of cells stained with a score of 0-3". The evaluation methods should be described in more detail.

We have edited the evaluation methods in our manuscript, which wrote “According to the staining intensity, we classified KOR protein staining as follows: 0 = absent expression, 1 = weak expression, 2 = moderate expression, 3 = strong expression. In addition, we determined the percentage of positive tumour cells staining with a score of 0 - 100. The two scores were multiplied to produce a weighted score for each case. Theoretically, the scores ranged from 0 (0% of cells staining) to 3 (100 × 3/100).” Please see Materials and Methods section, line 49 - 59, page 7.

3. The clinicopathological parameters 64 cases of fresh tumour tissues should also be collected and used for analysis.

The clinicopathological parameters of fresh tumour tissues have already collected according to your suggestion. You will find the results in Table 1.

4. ROC curves should be constructed.

The ROC curve was constructed and the result showed that KOR mRNA expression yielded AUC of 0.745, for the detection of HCC patients. You will find the results in Fig 1C.

5. The potential function and mechanism of KOR in HCC should be discussed.

We have made changes according to your suggestion. You will find changes in Discussion section, line 4-17, page 16.

6. It is suggested that Fig 1C and 2A should be presented with higher magnification.

We have made changes according to your suggestion. You will find changes in Fig 2A and Fig 3A.
Comments of Shah Walayat (Reviewer 2):

Interesting work about KOR expression in HCC both at protein and mRNA level. However in addition to the extensive editing of English, certain rearrangements in the results section would improve the presentation of the article. For example, from abstract and results at first glance it looks like both proteins and mRNA levels were determined in FFPE tissues which is not the case. So better to mention the main results of IHC first and then followed by real time PCR results from fresh tissue for confirmation of mRNA levels of KOR. Further no clinicopathological data is available for fresh tissue samples patients and also no IHC was performed in the fresh tissue. This area need further improvement. The term peripheral normal tissue may be replaced with adjacent normal tissue throughout the document to avoid confusion.

In the results "KOR is down-regulated in human HCC" both mRNA expression of 64 samples and IHC of 174 FFPE tissue is mentioned together. As suggested before the mRNA expression results should be placed at the end of the IHC results just for validation purpose. The same comments stand for the discussion section as well.

Answer:

Thank you very much for your kindly suggestions.

The samples used for IHC were collected from 174 patients who had undergone curative resection for primary HCC between 2003 and 2006 with the availability of follow-up data for at least 5 years. Fresh tissue samples collected from 64 patients who had undergone curative resection for primary HCC between May and July in 2016 would be beneficial to the reliability of PCR results, wherever these samples could not be available for a long time follow-up. Therefore, no IHC was performed in the fresh tissue. The clinicopathological parameters of fresh tumour tissues have already been collected according to your suggestion. You will find the results in Table 1.

Changes have already been made in our manuscript according to your suggestion. Please see Abstract section, line 16-17, line 31-32, page 2; Materials and Methods section, line 21-22, line 26-27, page 6.

Comments of Ryoji Yoshida, D.D.S., Ph.D (Reviewer 3):

This is a well written and easy to read manuscript, which aims to evaluate the clinical significance of κ-opioid receptor (KOR) in patients with hepatocellular carcinoma (HCC).

The authors demonstrated that KOR expression was significantly decreased in HCC tissues, and KOR expression status was one of the useful prognostic markers in patient’s prognosis. They
concluded that KOR might be a potential tumor suppressor in HCC progression and could provide a therapeutic target for HCC treatment.

Comments:

1. First, names of manufacturers with their locations (city and state/country) should be given for the first time only in the manuscript. Please check the Material & Methods section (e.g. p7, line 7 and line 54).

2. Please transfer your tables from the text of manuscript to the back of the figures. The authors should provide tables and figures as the separate data when they submit the manuscript.

3. There is no information about the management policy of the patients. The authors should describe how they follow-up their patients.

4. Since the grade of liver failure is important to decide the treatment of HCC, please show the Child-Pugh Score of study cohort as patient's characteristics.

5. In immunohistochemical studies, the authors should provide the photographs of hematoxylin and eosin (H&E) staining in order to facilitate the understanding pathological conditions.

6. In Table 1, KOR expression status significantly correlated with tumor differentiation grade. Please describe this point in discussion section.

Answer:

Thank you for your suggestion.

1. First, names of manufacturers with their locations (city and state/country) should be given for the first time only in the manuscript. Please check the Material & Methods section (e.g. p7, line 7 and line 54).

This manuscript has been edited according to your kindly advices. Please see the Material and Methods section, line 16-17, page 7; line 16-17, page 8.

2. Please transfer your tables from the text of manuscript to the back of the figures. The authors should provide tables and figures as the separate data when they submit the manuscript.
I put the tables in the manuscript according to the rules of “Preparing your manuscript” in BMC cancer, which wrote “Tables should NOT be submitted as figures but should be included in the main manuscript file”.

3. There is no information about the management policy of the patients. The authors should describe how they follow-up their patients.

The management policy of the patients has been mentioned in the “Ethics approval and consent to participate” part. Related details have already added in the Materials and Methods section, line 41-49, page 6.

4. Since the grade of liver failure is important to decide the treatment of HCC, please show the Child-Pugh Score of study cohort as patient's characteristics.

Related changes have been made in our manuscript. Please see Table 1, 2, 3, 4.

5. In immunohistochemical studies, the authors should provide the photographs of hematoxylin and eosin (H&E) staining in order to facilitate the understanding pathological conditions.

We have made changes according to your suggestion. Please see supplementary figure 1 in Additional file 1.

6. In Table 1, KOR expression status significantly correlated with tumor differentiation grade. Please describe this point in discussion section.

We have made changes according to your suggestion. Please see Discussion section, line 19-24, page 15; line 11-17, page 16.

Comments of Jessica Gilbert (Reviewer 4):

1. This manuscript indicated significance in a previously unknown relationship between KOR and HCC, which appears to be rather novel.

2. Why was KOR chosen as the focus receptor versus others?
3. Language and grammatical errors must be addressed for readability purposes. For example, please consider revision of language in the Introduction (Pg. 3, Paragraph 1, Sent. 5) to: "Therefore, investing a valuable biomarker to better evaluate the diagnosis and prognosis of HCC patients will be beneficial in the guidance of treatment and inhibition of metastasis". The use of the Oxford comma can be deemed as beneficial in grammatical setting such as: (Pg. 3, Paragraph 2, Sent. 5) "pain regulation, emotional tone "," and cognitive functions".

4. When using phrases such as "other studies" and "previous studies", make a note to describe in more detail how they compare to the present findings within your study.

5. In the Materials and Methods section, when describing the HCC patients and tissue specimens, were there any contradicting or pre-existing conditions within the population of patients that could account for error in the outcomes?

6. In the sub-section, "IHC analysis of KOR" of the Materials and Methods section states the staining intensity and percentages of cells stained on a score of 0-3. Quantified data may need elaboration for a more thorough description of the scores.

7. For Fig. 1 (C) and Fig. 2 (A), the magnification may need enhancement. Also, the magnification must be mentioned in Materials and Methods.

8. When referencing other authors, always cite the original and current manuscript, as well and still revise any original quote used.

Answer:

Thank you for your suggestion.

1. This manuscript indicated significance in a previously unknown relationship between KOR and HCC, which appears to be rather novel.

Thanks for your kindly comments.

2. Why was KOR chosen as the focus receptor versus others?

Studies of MOR expression and cancer have received the lion's share of attention in recent years. Lu and his colleagues (Lu J. Oncol Rep, 2013, 30(4): 1731-1738) have demonstrated that downregulation of MOR is able to inhibit both in vivo and in vitro human liver cancer progress. In addition, Tang and his colleagues (Tang B. Int J Oncol, 2013, 43(4): 1281-1290.) have shown that DOR is highly expressed in HCC and upregulation of DOR promotes cancer progression both in vivo and in vitro experiments. However, the expression profile and potential role of KOR in HCC remains unknown. Therefore, we choose KOR for investigation.
3. Language and grammatical errors must be addressed for readability purposes. For example, please consider revision of language in the Introduction (Pg. 3, Paragraph 1, Sent. 5) to: "Therefore, investing a valuable biomarker to better evaluate the diagnosis and prognosis of HCC patients will be beneficial in the guidance of treatment and inhibition of metastasis". The use of the Oxford comma can be deemed as beneficial in grammatical setting such as: (Pg. 3, Paragraph 2, Sent. 5) "pain regulation, emotional tone"," and cognitive functions".

We have made changes according to your suggestion. Please see Introduction section, line 16-22, line 41, line 45-46, page 4.

4. When using phrases such as "other studies" and "previous studies", make a note to describe in more detail how they compare to the present findings within your study.

We have made changes according to your suggestion. Please see Discussion section, line 12-14, page 13. Introduction section, line 31-33, page 4, “other studies have reported that opioids can induce apoptosis in serval cancer cells, such as lung cancer, colon cancer and breast cancer”, the purpose is to introduce the researches of opioids and to elicit the background information about opioid receptors.

5. In the Materials and Methods section, when describing the HCC patients and tissue specimens, were there any contradicting or pre-existing conditions within the population of patients that could account for error in the outcomes?

Before we started to collect HCC patients for our study, we have made precise inclusion criteria with a distinct pathological diagnosis with an absence of anticancer therapy prior to surgical resection or distant metastasis, and the availability of follow-up data. In our cohort study, we haven’t found such contradicting or pre-existing conditions so far.

6. In the sub-section, "IHC analysis of KOR" of the Materials and Methods section states the staining intensity and percentages of cells stained on a score of 0-3. Quantified data may need elaboration for a more thorough description of the scores.

We have edited the evaluation methods in our manuscript, which wrote “According to the staining intensity, we classified KOR protein staining as follows: 0 = absent expression, 1 = weak expression, 2 = moderate expression, 3 = strong expression. In addition, we determined the percentage of positive tumour cells staining with a score of 0 - 100. The two scores were multiplied to produce a weighted score for each case. Theoretically, the scores ranged from 0 (0% of cells staining) to 3 (100 × 3/100).” Please see Materials and Methods section, line 49 - 59, page 7.

7. For Fig. 1 (C) and Fig. 2 (A), the magnification may need enhancement. Also, the magnification must be mentioned in Materials and Methods.
We have made changes according to your suggestion. You will find changes in Fig 2A and Fig 3A, Materials and Methods section, line 9-12, page 8.

8. When referencing other authors, always cite the original and current manuscript, as well and still revise any original quote used.

We have made changes according to your suggestion. Please see Introduction section, line 19-22, page 5; Discussion section, line 32-35, page 13.