Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8141-review.doc).

Title: Clinical significance of nerve growth factor and TrkA signaling pathway in intrahepatic cholangiocarcinoma

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The manuscript has been improved according to the suggestions of reviewers:
Revision has been made according to the suggestions of the reviewer point to point.

Reviewer 1.

Question: Dear Authors, thank you for submission of this highly interesting manuscript. Especially the high quality in many parts supports acceptance of this paper. Just a minor comment: Please move 4 Sentences from results (Correlation between NGF, TrkA and overall survival rates) to method section of the manuscript: The relationship between.... (following 2 sentences) and In addition, multivariate analysis... (following two sentences). Instead you can summarize the results related to Tab 3 in a short paragraph. Congratulations! Sincerely Reviewer

Answer: Thank you for your comments. We have removed the mentioned sentences and summarize results to Tab 3.

Reviewer 2

(i)**Question**: In the Introduction the authors state that ?the relation between NGF and CCA has not been reported in clinical study? although in 2010, Xu LB et al. in their article "Nerve growth factor-beta expression is associated with lymph node metastasis and nerve infiltration in human hilar cholangiocarcinoma" (World J Surg 2010; 34:1039-45), they report that Nerve growth factor-beta was investigated by immunohistochemistry in samples from 28 cases of hilar cholangiocarcinoma and they found that Nerve growth factor-beta might promote lymph node metastasis and nerve infiltration in human hilar cholangiocarcinoma. The authors must comment on this article in their discussion section.

Answer: Thank you for your advice. We have revised our manuscript, and added comments of this referrence in our new manuscript.

(ii) **Question**: Below the horizontal line of Fig. 2, they must include patients at risk, in 10 months follow up interval.

Answer: We have updated Fig 2 and provided the patients number at risk. Thank you for your professional advice.

Reviewer 3

Major points:

Quesetion: 1. Discussion is quite rough and superficial, and also is not sufficient. Discussion is not a repeat of Results. Author should discuss some results in detail, such as 1) other publication

on NFG-TRKA as a biomarker or prognostic factor in other cancers, 2) on involving proliferation and invasion, the potential mechanism involved, based on present results. 3) Basic knowledge on NFG, TRKA and NFG-TRAKA signal pathway, their multiple functions, potential links with tumorigenesis, and significance of present results

Answer: Thank you for the instruction. According to the recommendations, we have rewrote our manuscript, including (1) discussing NGF-TrkA role in other cancers as biomarker;(2) potential mechanism of tumor progression and (3)association between signaling pathway and tumorgenesis.

Quesetion: Results should be precise and easy to follow. Some description in Results can be in Materials and Methods, and some in Results can be in Discussion. Author should reconsider the writing structure in both Results and Discussion

Answer: We have deleted the impropriate part of Results and moved some parts to Materials and Methods or Discussion. Thank you for the advice.

Question: Author gave a conclusion that both high expression of NGF and TRKA could be an independent prognostic factor of IHCC. According to author's grouping way, high expression of NGF is 38% and high TRKA expression is 28%. How many percent in both high expression of NGF and TRKA ? (At least less than 28%), and what is the significance as a prognosis factor in the low percent of high expression of NGF-TRKA?

Answer: Thank you for this professional comment. The percent of both high expression of NGF and TrkA was 15.6%(13/83), and we had supplemented this into the Results part. Firstly, we think the percent of higher expression of NGF-TrkA was not relatively low. In breast cancer, HER2 positivity was also only about 20 percent. Secondly, we think the significance of prognostic factor has no direct relation with percent of positivity. In Kaplan–Meier method, too lower percent of NGF/TrkA would cause higher P-value, which was the only criterion to judge significance. In our study, we think the percent of NGF/TrkA was acceptable and it had no influence of the significance as a prognostic factor.

Question: In Fig 2C, it seems the result is obtained from multivariate analysis of NGF+TRKA+(both high) vs NGF-TRKA-(both low). There should be four situations: both high expression, both low expression, only NGF high, only TRKA high. Author should provide these data and show how to analyze with multivariate analysis, such as, both high expression vs all others.

Answer: Thank you for this comment. It was our fault that the manuscript lacked detailed explanation. Actually, "NGF-TRKA-" in the former manuscript means all the other three situations except NGF+TRKA+(both high). We have corrected that and named it "non-NGF+TRKA+" in the updated manuscript. We analyzed the overall survival rate between NGF+TRKA+ and non- NGF+TRKA+ group by univariate analysis with Kaplan-Meier method. Thank you again for pointing this.

Question: Figure 3 showed the results on AKT and ERK, but less description in manuscript. Author should include the information on the results in Introduction, Results and Discussion, especially the significance in involvement in IHCC progression through NGF-TRKA signal pathway

Answer: Thank you for this advice. We have supplemented the correlation between signaling pathway and IHCC progression in the new manuscript, mainly in the Introduction and Discussion part.

Minor points .

Question: 1. Page 7, first line: The description "The samples were divided into positive and negative groups according to the average score" is not suitable. If author insists in grouping based on average score, " > average score and < average score" or "high expression and low expression"

Answer: Thank you. We have changed the description as "high expression and low expression". Thanks for the recommendation.

Question:2. Similar in page 9, "NGF was observed positively expressed in 38.3%(28/60) IHCC samples while TrkA was overexpressed in 28.3%(17/60) samples", it should be revised, such as, "The NGF expression more than average score was observed in …."

Answer: Thanks. We have revised all the manuscript as the instruction.

Question :3. It is necessary to descript the results precisely and in detail. For example, in page 9, "From table1, we can see that NGF was significantly associated with differentiation (P=0.024)." From Table 1, well differentiation has more case with high expression and poor differentiation has less case with high expression. Author should give precise description on the results.

Answer: Thanks for this professional advice. We have revised this point and described results more precisely.

Question :4. In page 10, in the result on multivariate analysis, please give a RESULT of the analysis

Answer: Thanks. We have added results on the multivariate analysis in the new manuscript. Thanks for pointing this.

Sincerely yours,

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