Author's response to reviews

Title: The Expression of Beclin-1, an Autophagic Gene, in Hepatocellular Carcinoma Associated with Clinical Pathological and Prognostic Significance

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Author's response to reviews: see over
Dear editor:

Thank you for your e-mail!

I appreciate you very much for your revision. My manuscript “The Expression of Beclin-1, an Autophagic Gene, in Hepatocellular Carcinoma Associated with Clinical Pathological and Prognostic Significance” was revised according to the reviewers’ comments. All the changes made in the paper are highlighted in red color.

For your guidance, itemized response to each reviewer’s comments is appended below:

1. Reviewer: Guido Valente:

Major changes

1) In Materials and Methods, the authors have included determination of CD34 and microvessel count, but no results are reported about this tool. Since tumor angiogenesis and autophagy are strictly connected pathways, they should add these data.

Reply: In this revising paper, the authors add the content of CD34 antibody in methods, supplementary results include the association of Beclin-1 expression with MVD in HCC, and the effect of the both Beclin-1 and MVD on the prognosis of HCC patients.

2) Results: in page 15-16 the authors reported the results obtained by combining different markers on the basis of level of positivity; these data
are rather heavy to follow and should be shortened and resumed (as they made in Table 4, which should be lightened and included in the text).

Reply: In this revising paper, the authors rewrite the results obtained by combining different markers in page 15-16, which content have shortened and resumed.

3) Tables 2, 3 and 5 should be also included in the original text to help in understanding results.

Reply: The key results in Table 2, 3, and 5 have added in the original text for helping reader understanding that results.

4) The discussion is too much long; the authors should avoid to report the functional characteristics of each marker they used, since I think they are usually known by the readership of the journal.

Reply: In discussion of this revising paper, a portion of the description in the functional characteristics of each marker have been deleted.

5) Two papers should be cited in the text: Lee YI et al (PLos One 2013) and Zou M (Cell Sign 2012).

Reply: In this revising paper the authors cite two new references, in which one, [Zou M (Cell Sign 2012)], is reviewer recommended to us, and another is Pan B (Cancer Biother Radiopharm, 2013,28(8):573-8).
Minor changes

1) Several mistakes: for instance in page 9 "follolical" B cells change in follicular; page 19: PCNA is a proliferation and not apoptotic markers; Bcl2, Bax and survivin are apoptotic markers.

Page 19: PCNA has become the most reliable marker to determine the proliferative activity of tumor cells: this sentence should be erased.

Reply: Several mistakes have corrected.

In page 9 "follolical" B cells have changed in follicular;

In page 19: “the association of Beclin-1 expression in HCC with cellular proliferation, apoptosis related proteins such as PCNA and NET-1, and proliferation related proteins including Bcl-2, Bax and Survivin are therefore studied.” have changed in “the association of Beclin-1 expression in HCC with cellular proliferation related proteins such as PCNA and NET-1, and, apoptosis related proteins including Bcl-2, Bax and Survivin are therefore studied.”.

In page 19: PCNA has become the most reliable marker to determine the proliferative activity of tumor cells: this sentence has been deleted.

2. Reviewer: Shu-Feng Zhou

1) Have the authors collected the demographics and characteristics of the patients?
Reply: 103 patients were enrolled from Nantong Tumor Hospital, in which hospital the scope of diagnosis and treatment cover QiDong district in Nantong of China, there is a high incidence of hepatocellular carcinoma in that district defined by World Health Organize. So the authors researched patients have a higher relatively the demographics and characteristics of the patients.

2) The role of autophagy in tumorogenesis is controversial. In this study, the authors only observed the expression level of a couple of key regulators of autophagy, apoptosis and cell proliferation by immunohistochemistry. In order to verify the role of autophagy in tumorogenesis, more studies are needed.

Reply: Because HCC is a highly vascularized tumor that requires the formation of numerous blood vessels to receive sufficient blood supply to grow and proliferate. Consequently, angiogenesis is a crucial process in the development of HCC. In this revising paper, the authors add the researching content of microvessels detected by CD34 antibody. The supplementary results include the association of Beclin-1 expression with microvessel density (MVD), and the effect of the both Beclin-1 and MVD on the prognosis of patients. Spearman related analysis and Pearson related analysis all demonstrated Beclin-1 expression negatively related to MVD. The 5-year OS of patients with positive co-expression of two factors were investigated by using Kaplan-Meier analysis (Fig.7) and
Log-rank test (Table 4). The results indicated that the patients in Beclin-1higher with MVD lower co-expression were significantly higher 5-year OS than that either in Beclin-1lower with MVD higher co-expression or in Beclin-1lower with MVD lower co-expression (57.1% vs 21.2% or 31.3% P<0.01). Therefore, observing the expression level of Beclin-1 and MVD at the same time help to evaluate the prognosis of HCC patients.

3) According to the observations in this study, it is not convincing to draw the conclusion that the expression of beclin-1, an autophagic gene, in hepatocellular carcinoma associated with clinical pathological and prognostic significance.

Reply: Although the mechanism of autophagy in oncogenesis is complex, in this paper, the authors have observed that the expression of Beclin-1 in HCC was negatively correlated with cirrhosis background, Edmondson grade and vascular invasion, the prognosis of patients with higher Beclin-1 expression are significant better than that with lower Beclin-1 expression. And the expression of Beclin-1 was negatively correlated with proliferating related proteins (PCNA and NET-1), anti-apoptosis protein (Bcl-2) and MVD, whereas positively correlated with pro-apoptosis protein Bax, respectively. These results demonstrate the expression level of Beclin-1 may be a valuable prognostic marker of HCC.
4) It needs to describe more details about the statistical analysis method used in this study.

Reply: In Materials and Methods, the authors have added to describe more details about the statistical analysis method for helping reader understanding that results.

5) There are a number of studies on the role of autophagy in tumorogenesis. The authors need to discuss more on the multifarious functions of autophagy in tumorogenesis.

Reply: Yes, in this revising paper, the authors add to discuss more on the multifarious functions of autophagy in tumorogenesis. The role that autophagy plays in oncogenesis is double sided and context depends. In a tumor microenvironment, autophagy can serve as a means of temporary survival in response to metabolic stress; meanwhile, once the cellular stress results in continuous or progressive autophagy, cell death would follow, which should be regarded as a direct cell death execution pathway or a garbage disposal mechanism whereby cells preserve their viability for long-time survival still needs clarification. Currently, numerous studies have been conducted to determine the molecular mechanism of autophagy, to explicit the role that autophagy played in tumorogenesis.

6) The references need to be updated.
Reply: In this revising paper, updating two references are appended below:


Finally, I want to express my appreciation again for all your assistances, and thank you for your suggestions! I will appreciate you very much if you have time to send me a reply e-mail. If you still have any question, please feel no hesitation to contact with me.

Best wishes,

Sincerely yours,

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