Author's response to reviews

Title: LC3B globular structures correlate with survival in esophageal adenocarcinoma

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Version: 2
Date: 20 July 2015

Author's response to reviews: see over
Dr Takaaki Arigami
Editor
BMC Cancer

Re: MS: 1956898708171193
‘LC3 globular structures correlate with survival in esophageal adenocarcinoma’

Dear Dr. Takaaki Arigami,

Thank you for the opportunity to revise our manuscript. We have now addressed all of the reviewer’s comments below and have edited our manuscript accordingly. The new revision has been uploaded, with all changes highlighted in red.

We hope that you will now find this paper acceptable for publication in BMC Cancer.

Yours sincerely,

Dr. Sharon McKenna
Response to Reviewers comments (blue type)

Reviewer 1

Title: LC3B globular structures correlate with survival in esophageal adenocarcinoma
Version: 1
Date: 11 June 2015
Reviewer: HONGLEI CHEN
Reviewer's report: Minor Essential Revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: No

Report:

Although some papers about LC3B clinical significance in the esophageal carcinoma had been published, however, different staining patterns of LC3B in the esophageal adenocarcinoma has not been reported, the authors identified the clinical significance of a different staining patterns of LC3B in the esophageal adenocarcinoma. The methods are appropriate and well described, and are sufficient details provided to replicate the work. All the data are sound and well controlled. The discussion and conclusions are balanced and adequately supported by the data, if the discussion could be concise. The title and abstract accurately convey what has been found. The writing is acceptable.

However, I think that the quantification standard of three different staining patterns of LC3B is assertive, absent of enough evidence to support it. I suggest the author can perform the receiver operating characteristic (ROC) curve analysis with the respect to overall survival, and then determine the cutoff point for high and low expression of LC3B.

Response:

We have performed ROC. The graph is shown below.
The area under the curve is equal to 0.8 indicating that this scoring method (for counting globular structures) is an appropriate test. The cut off value to have a sensitivity and specificity of approximately 80% is around 4. We used this value to classify tumors as positive, if we count four structures or more. Less than four structures was classified as negative.

We have now commented on this method in the Methods ‘Quantification of Immunohistochemistry staining’ section page 8, and in the ‘Statistical analysis’ section page 8.

Reviewer2

Title: LC3B globular structures correlate with survival in esophageal adenocarcinoma
Version: 1
Date: 19 June 2015
Reviewer: Takaaki Arigami
Reviewer’s report: Major Compulsory Revisions

1) 104 naïve patients and 48 patients treated with neoadjuvant therapy were enrolled in this study. The authors should indicate the indication of neo-adjuvant therapy in Methods section.

The indication for neoadjuvant treatment is TMN stage. It is not indicated for metastatic disease. It is only indicated for patients who are candidates for curative surgical resection. This has been added to the Methods, ‘Patients’ section page 5

2) Please indicate the relationship between LC3B staining patterns and other clinicopathological findings such as depth of tumor invasion, lymph node metastasis, and distant metastasis in Table 3.
The correlation with lymph node metastasis is shown in Table 3. No correlation was found between LC3B staining patterns and depth of tumor invasion and distant metastasis.

3) In the present study, 48 patients receiving neo-adjuvant therapy were enrolled. How about the relationship between LC3B staining patterns and therapeutic effects of chemo-radiotherapy?

The LC3B staining patterns were only examined in relation to overall survival. We did not investigate the relationship between LC3B staining patterns and therapeutic effects of chemo-radiotherapy. As we had patients who received therapy and those that did not, this enabled us to investigate the effect of therapy on the LC3B expression patterns.

Unfortunately, we do not have the data to enable adequate analysis of therapeutic effect. The pre-treatment criteria for staging were primarily clinical criteria (i.e. MRI / tumour size). The biopsy prior to treatment is usually small in size and incorporates mainly the mucosal layer. All patients had surgical resection after chemo-radiotherapy. There was no further clinical staging to compare with pre-treatment. Staging at this stage is pathological. It is difficult therefore for us to compare between the two.

4) This study demonstrated that cytoplasmic LC3B pattern was significantly related with a favorable prognosis. On the other hand, globular staining pattern was correlated with a poor prognosis. These results means the inverse malignant behavior. The authors should discuss about these findings, including the oncological viewpoint, in Discussion section.

In the discussion, we have suggested that the cytoplasmic staining is analogous to a more normal cellular phenotype – and this may reflect the better prognosis. All normal cells will have the capacity to undergo autophagy and can demonstrate a punctate diffuse staining of autophagosomes.

Discussion page 19 is clarified as follows:

‘The origin of these morphologically distinct LC3 stained structures is unknown. The more diffuse staining in the cytoplasm may reflect soluble LC3B and basal physiological autophagic activity. If these cancer cells have a more ‘normal’ physiology – this may explain the better prognosis. In contrast, the LC3A/B globular pattern is an exclusive finding of aggressive malignant epithelial cancers and may reflect an exaggerated or aberrant form of autophagic activity. Further research is needed to investigate the nature of this structure, which is clearly linked to an aggressive tumor phenotype’.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I have no competing interests.