

Format for ANSWERING REVIEWERS

August 17, 2016



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 27905-Revised manuscript.docx).

Title: Inverse correlation between CD8+ inflammatory cells and E-cadherin expression in gallbladder cancer: Tissue microarray and imaging analysis

Author: Keita Kai, Masanori Masuda and Shinichi Aishima

Name of Journal: *World Journal of Clinical Cases*

ESPS Manuscript NO: 27905

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Responses for the reviewers

#Reviewer 1

It is an interesting study. But need to add the following points 1. Please evaluate the relationship between the tumor cells' expression of E-cadherin/CD8+ and Clinical TNM staging of gallbladder cancer (GBC). It would be better to illustrate the relationship between the expression of these indexes and the prognosis of the tumor. 2. This study would be more convincing to elucidate the prognostic significance of combining E-cadherin with CD8+ in gallbladder cancer (GBC).

Response: Thank you for your valuable comments. Our answers are following.

1. We have additionally performed analyses of relationship between E-cadherin/CD8+ and Clinical TNM staging of GBC. We have added the results of these analyses in RESULTS section and newly added the Figure 6 and 7 and added regarding discussion with new reference (Ref. #21) at DISCUSSION section of fourth paragraph. We would like to thank for the reviewer's reasonable comment.
2. We have investigated the correlation between the invasion of CD8+ T cells/expression of E-cadherin and the prognosis of GBC cases (disease free survival, overall survival and disease specific survival), and no significant relationship was found. This is documented in DISCUSSION section, third paragraph.

#Reviewer 2

There were major problems in this manuscript. 1. In this study, several labels of inflammatory cells were detected, such as LCA, CD3 and CD8. It was shown that these labels were significantly correlated with the expression of E-cadherin. But the author did not discuss the relationship between LCA, CD3 and the expression of E-cadherin. 2. The expression location of CD8 in the tissue was not identified. 3. In table 4, The median \pm SD of CD8+ cells was 580.0 ± 1154.5 . Did it meet normal distributions since standard deviation exceed median? 4. This study showed a significant inverse correlation between the number of infiltrating CD8+ cells at invasive areas and the expression of E-cadherin. But in the part of discussion, the author did not illuminate the significance of this phenomenon. Moreover, this study observed that relatively large numbers of acute inflammatory cells such as neutrophils and macrophages had infiltrated into invasive areas in GBC. What about other tumors?

Response: Thank you for your valuable comments. Our answers are following.

1. LCA labels all leucocytes and CD3 labels all T-lymphocytes, therefore CD8+ T-lymphocytes are involved in CD3+ cells and LCA+ cells. As shown Figure 4, the most significant data was CD8+ cells, therefore we demonstrated these data and discussed.

2. This study was based on tissue microarray analysis. Therefore, referring to the location of CD8+ cells is impossible.
3. The standard deviation is sometimes higher than median when the variation of the data among samples is large.
4. The discussion regarding CD8+ cells and E-cadherin expression was documented in sixth paragraph of DISCUSSION section. It is impossible to clarify the mechanism of this phenomenon by our results only. Further analyses are needed. Infiltration of neutrophils and/or macrophages are often observed in other site of cancer but it depends on case by case.

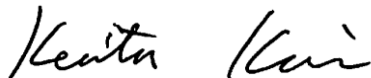
#Reviewer 3

The Author thoroughly introduced gallbladder clinical procedure and pathological conditions. The review has some clinical significance. So suggest to accepted it.

Response: No response is needed. We would like to thank the reviewer's time and effort.

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

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Keita Kai'.

Keita Kai, MD, PhD

Department of Pathology, Saga University Hospital

Nabesima 5-1-1, Saga City, Saga 849-8501, Japan

Tel: +81-952-34-3264; Fax: +81-952-34-2055

E-mail: kaikeit@cc.saga-u.ac.jp