Peer Review File

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To reviewer 1:

Comment 1: IHC staining images of CD8, HLA- class I and PD-L1 positive and negative images should also be included for readers to more clearly understand what was classified as positive and negative in this study and how good was staining.

Reply 1: As the reviewer mentioned, it is easier to understand if the immunostaining image for each antibody is shown, so the images of positive and negative cases for each antibody were added.

Figures (Fig. 2B and 2C) were added.

Comment 2: What was the correlation between KK-LC-1 expression and Ki67 expression in tumors? This needs to be determined to confirm if there is correlation between proliferation capacity of cancer cells and KK-LC-1 expression. The authors should also investigate the impact of Ki-67 staining (high) on prognosis in case of KK-LC-1 positive tumors if Ki67 staining is not high in all KK-LC-1 positive tumors.

Reply 2: The correlation between the expression of KK-LC-1 and Ki67 has not been statistically confirmed. In this time, KK-LC-1 expression tended to be localized to the basal site of the tumor tissue. Therefore, we conducted an additional analysis to investigate whether KK-LC-1 expression is strong on the site where tumor growth is active. Four representative cases with KK-LC-1 expression-positive lung squamous cell carcinoma were immunostained using Ki-67 antibody. Ki-67 expression was also localized on the basal site in all 4 cases analyzed, suggesting that KK-LC-1 expression may be increased on the basal site where tumor growth is active. However, it cannot be concluded that it is not analyzed in many cases. We corrected the wording in our text.

Changes in the text. Lines 2-5, 8-10 in Page 5, Lines 12-15 in Page 10, Lines 4-8 in Page 12 and Lines 8-10 in Page 15 were added.

To reviewer 2:

Comment 1: The conclusion that tumour with KK-LC-1 expression and the tumor infiltrating CD8+ T cells exhibited better prognosis (as also mentioned in the abstract) is misleading, as the driver behind this better survival would appear to be the presence of CD8+ cells, with very little effect (and possibly none) exerted by KK-LC-1 in terms of survival analysis. I thus have doubts as to the conclusions drawn in this paper and would suggest to rewrite to reflect the fact that a role for KK-LC-1 is not yet proven from these data.

Reply 1: As the reviewers pointed out, there is possibility that the good prognosis for patients with KK-LC-1-positive squamous cell carcinoma of the lung with tumor infiltrating CD8+ T cells is derived from the especially good prognosis of cases with tumor infiltrating CD8+ T cell. However, as mentioned in the discussion, there are some limitations in this analysis, and it is necessary to analyze with more cases in order to conclude. Therefore, we have corrected the wording in the text appropriately.

Changes in the text. Lines 7-10 in Page 5 were added.

Comment 2: The sentence in the abstract 'However, there was no difference in the prognosis with respect to KK-LC-1 expression regardless of expression of the HLA class I expression or the PD-L1 expression' is unclear and needs to be rewritten.

Reply 2: We have rewritten the text, as the reviewer pointed out.

Changes in the text. Lines 15-17 in Page 4 were added.

Comment 3: The scoring of areas of tumour which containing CD8+ cells rather than counting these cells, is unusual. This needs to be explained in ore detail, i.e. how were distinctions between CD8-positive and CD8-negative areas made, and how large were the minimum segments of tumor assessed and placed in categories by this method?

Reply 3: Various methods have been reported to evaluate tumor infiltrating CD8 + T cells. In a previous study (Ref. 10), we found that the ratio of the area of CD8-positive cells to the area in the tumor stroma was more reflected in the prognosis, so we adopted this method in this study. This method is based on the literature (Rakaee M, Kilvaer TK,

Dalen SM, *et al*. Evaluation of tumor-infiltrating lymphocytes using routine H&E slides predicts patient survival in resected non-small cell lung cancer. Hum Pathol 2018; 79: 188-98.).
Changes in the text. Lines 16-19 in Page 12 were deleted. Ref 22 was added.

Comment 4: Which threshold of staining intensity was used for HLA positivity?
Reply 4: Regarding the determination of whether or not the cancer cells were stained with HLA class I antibody, it was counted that the cancer cells were objectively and clearly stained with reference to the opinions of two skilled pathologists. Then, it was determined how much of the viable cancer cells were stained with HLA class I antibody. From the ROC curve, if 15% or more of the cancer cells were stained, it was judged to be positive.

Comment 5: Why was the percentage area of PD-L1 staining used rather than the more standard cell number percentage (TPS)?
Reply 5: In this time, we adopted the same area ratio as HLA class I and CD 8+ T cells. However, in reality, the commonly used number ratio and area ratio were almost the same.

Comment 6: In the results section the author mention with respect to KK-LC-1 expression that 'the specimens that exhibited staining in the nucleus of lung cancer cells were evaluated as positive (Fig. 3A), and those without staining were evaluated as negative (Fig. 3B)', while in the methods they also include cytoplasmic staining. Which is it?
Reply 6: As the reviewer pointed out, KK-LC-1 is expressed not only in the nucleus but also in the cytoplasm. In Fig. 3A, the cytoplasm is also stained with the KK-LC-1 antibody, so "cytoplasm" was added in the text.
Changes in the text. Line 20 in Page 9 was added.