TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

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Reviewer A

Submitted manuscript titled “Glypican-1 is a novel immunohistochemical marker to differentiate poorly differentiated squamous cell carcinoma from solid predominant adenocarcinoma of the lung”, by Yuichiro Kai, et al examined the clinical applicability of glypican-1 as a diagnostic marker differentiating lung squamous cell carcinoma from lung adenocarcinoma, using GEO dataset and immunohistochemistry. They reported that Glypican-1 is a good diagnostic marker for the diagnosis of lung squamous cell carcinoma. With high sensitivity and specificity, they were able to distinguish poorly differentiated squamous cell carcinoma from poorly differentiated lung adenocarcinoma. Despite the considerable interest in the present study, it’s my opinion that the article could be improved and require some additional information and correction that I will report in this revision.

1. How did the four pathologists handle the readings when the glypican-1 expression readings were inconsistent?

We added sentences in Methods.

‘If there were disagreements in pathological diagnosis, the pathological records were reviewed, and a consensus was reached after discussion.’ [Page 9: Line 140-142]

2. In discussion, Aviel-Ronen S et al. (20) and Tsuta K et al. (21) showed positive staining in 55% and 46.7% of lung squamous cell carcinoma, respectively. The past results of other authors are very different from those of the present authors. Describe why the present results differ from previous other authors' results (eg, antibody clone, immunohistochemistry autostainer, detection kit, tumor differentiation...).

→Aviel-Ronen S et al. and Tsuta K et al. reported the glypican-3 expression. However, we evaluated the glypican-1 expression. The difference between glypican-3 and glypican-1 seemed to cause the different results.

We are very sorry for confusing you with including glypican-3. Now, we delete the description of glypican-3 and focus the glypican-1.

We deleted sentences in Discussion.

‘Increased expression of glypican-3 has been reported in lung squamous cell carcinoma. Aviel-Ronen S et al. (20) and Tsuta K et al. (21) showed positive staining in 55% and 46.7% of lung squamous cell carcinoma, respectively, which indicated the difficulty of adopting glypican-3 in the differential diagnosis of lung squamous cell carcinoma.’

And added sentences in Discussion for answering the following question 3.

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The overexpression of glypican-1 has previously been reported in breast cancer (20), pancreatic cancer (21), and gliomas (22). [Page 14: Line 236-238]

3. Glypican-1 overexpression was also identified in pancreas cancer (Cancer Med. 2017 Jun;6(6):1181-1191.), breast cancer tissues (Cancer Res. (2001) 61:5562–9.) and ovarian malignant tumors (Clin Cancer Res. (2004) 10:5178–86.). In the discussion, it is necessary to comment that glypican-3 is difficult to use in distinguishing pancreas, breast and ovary cancer from lung squamous cell carcinoma.

→Do you indicate that '*glypican-1 is difficult to use in distinguishing pancreas, breast and ovary cancer from lung squamous cell carcinoma'* in your sentence?

Thank you for your advice.

We added sentences in Discussion.

'Moreover, glypican-1 is not useful for distinguishing specific metastatic lung cancers from lung squamous cell carcinoma because of the glypican-1 overexpression for breast cancer (20), pancreatic cancer (21), and gliomas (22).' [Page 17,18: Line 304-311]

4. In immunostaining for diagnosis, it is important that the antibody is verified in several different cohorts. There is no internal or external validation in the author's study. In particular, the immunohistochemistry for Glypican-1 has few studies. Are there any other studies using the glypican-1 antibody (rabbit polyclonal, 1:250, Proteintech, Cat# 16700-1-AP, RRID: AB_1851168) the author used?

→We (Amatya VJ et al.) already reported this glypican-1 antibody expression for mesothelioma and lung adenocarcinoma (12). Thus, the present study adopted this rabbit polyclonal antibody. There were no other studies of using this glypican-1 antibody for lung cancer, however, Saito T et al. reported this glypican-1 immunohistochemistry in glioblastoma.

We added sentences in Discussion.

'We adopted anti-glypican-1 antibody (rabbit-polyclonal, 1:250, Proteintech, Cat# 16700-1-AP, RRID: AB 1851168), which we and Saito T et al. used in the previous study (12, 24), respectively.' [Page 16: Line 269-272]

5. The authors identified only the rate of expression of immunohistochemistry and did not comment on the intensity of expression. Is there any difference in the intensity of glypican-1 expression in lung squamous cell carcinoma or lung adenocarcinoma?

→In lung squamous cell carcinoma, all 63 cases showed the strong intensity for glypican-1 expression. In lung adenocarcinoma, 2 cases were positive for glypican-1 expression and the intensity was weak.

We modified and added sentences in Results.

'All 63 (100%) cases of lung squamous cell carcinoma showed strong positivity for glypican-1 expression.' [Page 12: Line 188,189]
'and the intensity was weak.' [Page 12: Line 191,192]

6. In pathological diagnosis, patch p40 or CK5/6 expression appears in many poorly differentiated lung adenocarcinomas. Was patch Glypican-1 expression not observed in poorly differentiated lung adenocarcinoma?
→As you mentioned, among 60 cases of poorly differentiated lung adenocarcinoma (solid predominant lung adenocarcinoma), 5 cases showed patch (+1) p40 and CK5/6 expression, respectively. On the other hand, only 2 cases showed patch (+1) glypican-1 expression. The specificity of glypican-1 was better than that of p40 and CK5/6, but not perfect. Thus, combinations of useful immunohistochemical markers were essential.
We already mentioned patch glypican-1 expression in Results.
'Only 2 out of 60 (3.3%) lung adenocarcinoma cases were positive for glypican-1, both showing focal expression with 1+ immunohistochemical score (Supplementary Figure 1)' [Page 18: Line 308-310]

Reviewer B
In this paper, the authors describe glypican-1 as a novel immunohistochemical marker of squamous cell carcinoma of lung, demonstrating its high sensitivity, specificity, and accuracy in distinguishing squamous cell carcinoma from adenocarcinoma of the lung. The paper is very well written and addresses a clinically relevant area. The authors have appropriately acknowledged that the data need to be validated in other laboratories.

The manuscript needs to clarify whether all samples of squamous cell carcinoma and adenocarcinoma were poorly differentiated. If not, it will be helpful to know the % of tumors that were poorly differentiated.
→All samples we adopted were poorly differentiated lung squamous cell carcinoma and solid predominant lung adenocarcinoma.
We already mentioned all samples in Methods.
'We retrieved the surgical specimens of 63 poorly differentiated lung squamous cell carcinoma and 60 solid predominant lung adenocarcinoma from the tissue archives of the Department of Pathology, Hiroshima University.' [Page 9: Line 131-133]