Peer Review File

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Reviewer A

Comment 1: Patients with ALK+ lung adenocarcinoma typically present at advanced stages, and a solid & signet ring cell morphology is one of characteristic features of ALK+ lung adenocarcinomas; thus, lymph node metastases with signet ring cells are not rare in this setting (at least in the Western population).

Reply 1: Thanks very much for your kind and useful comment. After carefully reading your comment and reviewing the related literatures again, we agreed that lymph node metastases with signet ring cells are not rare, and this is not enough innovative in the manuscript.

The innovation of the paper is repositioned. After modification, the main innovation point is: a case of ALK-positive metastatic pulmonary adenocarcinoma with signet ring features (PASRF) with polygonal cell morphology to supraclavicular lymph node was reported. Two morphology tumor cells were observed. Immunohistochemically, two morphology tumor cells were diffusely positive for TTF1, CK7, Napsin A, most were p40, p63, CK5/6 positive, CK20 and CDX2 were negative. Co-expression of TTF-1, CK7, Napsin A and p40, p63, CK5/6 was observed in the two morphology tumor cells. Alcian blue-periodic acid-Schiff (AB-PAS) staining showed diffused intracytoplasmic mucin in the two both cells. Further, ALK rearrangements were detected by ALK (clone D5F3) immunohistochemical assay and fluorescent in situ hybridization (FISH). A final diagnosis of ALK-positive metastatic PASRF with polygonal cell morphology to supraclavicular lymph node was made. The patient was treated with ALK inhibitor Alectinib, and in good condition with tumor decreasing in size. It is important to notice PASRF with polygonal cell morphology in nodal metastasis so that the patients could benefit from ALK-targeted therapy.

We have readjusted the innovation of the paper and revised the great length in the manuscript.

Reviewer B

Comments to author

This paper reported a case of ALK-positive lung adenocarcinoma with signet ring cell features which metastasized to a lymph node. Lymph node metastasis of signet-ring cell carcinoma may be misdiagnosed as other metastatic cancer, especially gastric cancer. Therefore, it is important to notice signet-ring cell features of ALK-positive lung cancers in nodal metastasis so that the patients could benefit from ALK-targeted therapy.

It is necessary that author should improve some points.

Comment 1: p3: Primary lung adenocarcinomas with signet ring cell features are rare in pulmonary cancers. However, adenocarcinoma with signet ring cell features frequently metastasized lymph node (p9: reference, 5). In addition, ALK-positive lung cancers frequently metastasize for lymph node. Therefore, I don't agree that lymph node metastasis of ALK-positive cancers with signet-ring cell features is extremely rare.

Reply 1: Thanks very much for your kind and useful comment. After carefully reading your comment and reviewing the related literatures again, we agreed that lymph node metastases with signet ring cells are not rare, and this is not enough innovative in the manuscript.

The innovation of the paper is repositioned. After modification, the main innovation point is: a case of ALK-positive metastatic pulmonary adenocarcinoma with signet ring features (PASRF) with polygonal cell morphology to supraclavicular lymph node was reported. Two morphology tumor cells were observed. Immunohistochemically, two morphology tumor cells were diffusely positive for TTF1, CK7, Napsin A, most were p40, p63, CK5/6 positive, CK20 and CDX2 were negative. Co-expression of TTF-1, CK7, Napsin A and p40, p63, CK5/6 was observed in the two morphology tumor cells. Alcian blue-periodic acid-Schiff (AB-PAS) staining showed diffused intracytoplasmic mucin in the two both cells. Further, ALK rearrangements were detected by ALK (clone D5F3) immunohistochemical assay and fluorescent in situ hybridization (FISH). A final diagnosis of ALK-positive metastatic PASRF with polygonal cell morphology to supraclavicular lymph node was made. The patient was treated with ALK inhibitor Alectinib, and in good condition with tumor decreasing in size. It is important to notice PASRF with polygonal cell morphology in nodal metastasis so that the patients could benefit from ALK-targeted therapy.

We have readjusted the innovation of the paper and revised the great length in the manuscript.

Comment 2: p5: The tumor biomarkers of POSROMA and PREROMA are not commonly used. The authors should explain these biomarkers in details.

Reply 2: Thanks for your kind suggestions. ROMA index has some value in predicting ovarian cancer. POSROMA refers to postmenopausal ROMA index, and PREROMA refers to premenopausal ROMA index. It has some value in predicting ovarian cancer (J Clin Lab Anal. 2019 Jan;33(1): e22624.). Its predictive value for lung cancer has not been reported. Our intention is to show the objective test results to the readers. Meanwhile, there may be some misunderstanding for readers showing the two items in the manuscript. In the light of this point, we consider deleting tumor biomarkers of POSROMA and PREROMA.

Changes in the text: we have deleted POSROMA and PREROMA in our manuscript (see Page 7, line 119).

Comment 3: p6 and 7: In immunohistochemistry, authors showed that the tumor cells were positive for p63 and CK5/6. p63 sometimes expressed in ALK-positive adenocarcinoma. However, the immunohistochemical analysis of p40 should be performed to identify squamous differentiation, because p40 exhibits higher specificity than p63.
Reply 3: Thanks for your useful suggestions. Diffused co-expression of TTF-1 and p63 was observed in almost half of the PASRFs (Yoshida A, Tsuta K, Watanabe S, et al. Frequent ALK rearrangement and TTF-1/p63 co-expression in lung adenocarcinoma with signet-ring cell component. Lung Cancer 2011;72:309-15.). According to your suggestion, immunohistochemical analysis of p40 has been done. The staining shows most of both two tumor cells are p40 positive, which is beyond our expectation. As you mentioned, p40 exhibits higher specificity to identify squamous differentiation. AB-PAS staining was further performed to confirm the extensive intracellular mucus in the two morphology of tumor cells. A final diagnosis of metastatic PASRF with polygonal cell morphology to supraclavicular lymph node was made.
Changes in the Figure: we have added figures 2F of p40 immunostaining. (see Page 15, Figure 2F).

Comment 4: p7: In digestive tract, primary tumor cells are usually TTF1(-), CK20(+) and CDX2(+).
Reply 4: Thanks very much for your careful and patient comments. We totally agree with the point that primary tumor cells of digestive tract are usually TTF1(-), CK20(+) and CDX2(+). We made a mistake in the first manuscript and have revised it in this manuscript.
Changes in the text: we have revised it in this text（see Page 11, line 213）.

Comment 5: p11 Figure legend: In figure 1(F), circle is blue, not red.
Reply 5: Thanks for your kind reminder. We have revised red to blue accordingly.
Changes in the Figure legend: we have revised in Figure 1F legend (see Page 15, Figure legend).

Comment 6: p12: In figure 2 E, the stromal cells surrounding cancer cells are positive of p63. I suppose that p63 immunohistochemical staining probably failed, because negative control (stromal cells) is positive. I think that there are several possible causes, such as non-specific reactions, too long of DAB reaction time, and unsuitable antigen activation. Therefore, the authors should perform re-staining of p63 (or p40).
Reply 6: Thanks for your professional advice. According to your suggestions, we optimized the experimental conditions and p63 immunohistochemical staining was repeated, also p40 staining was performed. The results showed that most of the two tumor cells are definitely positive for P40, P63, and the stromal cells surrounding cancer cells are negative.
Changes in Figure 2: we have added figures of p40 and replaced p63 in Figure 2F and 2G, respectively. (see Page 15, Figure 2F, 2G). Further discussion and AB-PAS

experimental are carried out (see Page 10, Line 189-200)

Comment 7: p12: In figure 2 B, magnification is too small to distinguish between polygonal cells and signet ring cells.
Reply 7: Thanks for your kind suggestions. We have replaced the corresponding figure with greater magnification. In Figure 2B, signet-ring cells and polygonal cells are labelled with arrow and arrowhead, respectively.
Changes in Figure 2: we have replaced a greater magnification in Figure 2B (see Page 15, Figure 2B).

Comment 8: p12: In figure 2 C, magnification is too small to observe mitotic figures.
Reply 8: Thanks for your kind suggestions. We have labelled mitotic figure with greater magnification in Figure 2B, where mitotic figure could be observed.
Changes in Figure 2: we have replaced a greater magnification in Figure 2B (see Page 15, Figure 2B).

Comment 9: p12: figure 2 A to F: All figures contain scale bar. Magnifications of all figures are different, but the scale bar is the same. The authors should adjust the scale bars and mentioned it in the legend.
Reply 9: According to all your comments on this manuscript, we have added and replaced corresponding figures, all scale bars are adjusted.
Changes in Figure 2: we have replaced figures and adjusted all scale bars (see Page 15, Figure 2).

Comment 10: p13: In figure 3B, the magnification is too small to observe ALK fusion signal.
Reply 10: Thanks for your kind suggestions. We have changed greater magnification in Figure 3B, and ALK split signal is labelled with yellow arrows.
Changes in Figure 3: we have replaced a greater magnification and labelled ALK split signal with yellow arrows in Figure 3B (see Page 15, Figure 3B).