Peer review file

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Reviewer A

In this case presentation, the authors reported a case of metastatic lung adenocarcinoma harboring a novel ALK fusion (Chr1 95026203 -ALK intergenic fusion). The reviewer thinks it would be worth reporting such a rare case, however, several important data are missing as summarized below.

Comment 1: Activating mechanism for the novel ALK fusion (Chr1 95026203 -ALK intergenic fusion) should be analyzed and described. Usually, a partner gene (such as EML4) has a domain (such as coiled-coil domain) that enables dimerization of the fusion gene.

Reply 1: Thanks for your reminding. More and more reports are talking about intergenic fusion of ALK, mainly about the appropriate detecting method, activating mechanism and response to ALK inhibitors in patients carrying it (1,2). As suggested in Li et al., 2020, chromothripsis may contribute to the complex rearrangements, for which the actual fusion partner joins to an intergenic region and then on to the kinase gene but the intergenic region is then removed during transcription (case 6, intergenic fusion detected by DNA, while EML4-ALK detected by RNA). Alternative splicing may also be responsible (Case 11), that the exon of the upstream gene was spliced to exon of driver gene in the transcript. As a result, further RNA-level or protein-level validation was widely recommended before adopting target therapy. The intergenic ALK fusion in this case was validated by IHC, without results of RNA since there's no residual sample for RNA sequencing. Combing the partial response to crizotinib in two cases in Li et al., 2020 (intergenic- ALK fusion verified by IHC), we considered Chr1 95026203 -ALK as a targetable fusion variant and patients with it might benefit from ALK inhibitors.

Changes in the text: Page 3-4, Line 57-59.

1. Li W, Liu Y, Li W, Chen L, Ying J. Intergenic Breakpoints Identified by DNA Sequencing Confound Targetable Kinase Fusion Detection in NSCLC. Journal of Thoracic Oncology. 2020 Jul;15(7):1223–31.

2. Chen X, Zhao G, Zhong P, Zhang M, Chen R, Zhang D. Chr2 30297612-ALK, A Novel Intergenic Fusion With Exon18 of ALK, Responds to Crizotinib. Clinical Lung Cancer. 2020 May;S1525730420301376.

Comment 2: ALK IHC and HE staining of the tumor should be shown as a figure.

Reply 2: Please see the Figure 2 of H&E staining and ALK IHC, based on specimens sent for IHC validation.
Changes in the text: Figure 2(B)&(C).

Comment 3: Please describe the ratio of tumor cells in the biopsied specimen. This data is essential to evaluate if 3.86% of ALK-fusion abundance is low or not.

Reply 3: The specimen sent for DNA-based NGS was paraffin wax film, which was unable to confirm the ratio of tumor cells. While, based on specimen sent for IHC validation (Figure 2), the ratio of tumor cells is 5%.
Changes in the text: None.

Comment 4: The reviewer considers that the presented CT imaging (Figure 1A) is not GGO. This should be classified into a pure solid nodule.

Reply 4: Sorry for the misleading imaging, we added figures of different sections from the same CT scanning, hope it could present GGO clearly.
Changes in the text: Figure 1.

Comment 5: Smoking status, PET data, the image data that were taken 6 months before, CEA and other tumor marker data, IHC for TTF-1 or others, and treatment outcomes (with chemo) should be added.

Reply 5: Thanks for your reminding, the available data are completed in the text based on the latest medical records. While we couldn't obtain imaging of 6 months ago since it was not performed in our hospital. Trend of CEA was given in Figure 3.
Changes in the text: Page 2, Line 29 & 34-37, and Figure 3.

Reviewer B

The authors reported on a novel ALK fusion. However, it is the only new knowledge that we can acquire from this report. There is no molecular information about whether or not this fusion is oncogenic. Also, there is no data whether this fusion is sensitive to ALK-TKI or not. The authors should also comment on the potential role of Chr1 95026203.

The authors show in Figure 1 the irregular-shaped nodule. We cannot confirm that there is GGN component from this figure. The authors should show a thin-slice CT image that clearly shows the GGN component. Furthermore, this patient had multiple lung nodules and it is difficult to conclude that the metastatic lymph node was related to any GGN nodule. Overall, I think it is hard to demonstrate any correlation between this fusion and GGN just from analyzing the biopsy specimen of the metastatic lymph node.

Reply: We totally agree with you on this point. Although high concordance of ALK fusion (98%) was revealed between primary and the lymph node metastasis (3), heterogenous could still exist. We could only speculate that, the nodule with GGO component might also carry this novel intergenic ALK fusion and increase the evidence of ALK fusion in GGO-featured lung cancer.

Changes in the text: Page 4, Line 73-75.

3. Hou L, Ren S, Su B, Zhang L, Wu W, Zhang W, et al. High concordance of ALK rearrangement between primary tumor and paired metastatic lymph node in patients with lung adenocarcinoma. J Thorac Dis. 2016 Jun;8(6):1103–11.

Reviewer C

Chen and colleagues report a novel intergenic fusion of Chr 195026203-ALK in NSCLC cancer patient with radiological manifestation in form of ground-glass opacity. Theoretically intergenic-breakpoint fusions, in which one or both genomic breakpoints localize to intergenic regions, are mostly unlikely to be functional as there are no chimeric full-coding transcripts, and thus, no chimeric fusion proteins are produced. There are already at least 28 intergenic ALK-rearrangements identified and their functional significance is still not established. However, with three of them were reported as responding to Crizotinib and one responding to Crizotinib and Alectinib (1). The article contributes to the current knowledge of intergenic ALK-rearrangements as they represent a subtype of gene fusions with potential oncogenic functions (2). Activation of ALK gene, which expects not to be longer active in adults, especially containing exon 20-29, can cause functional transcript (chimeric RNA) and its product protein was demonstrated by ALK-IHC.

Another important message is the relation of this new fusion to the radiological features of the disease in form of ground-glass opacities (GGO).

As the authors mention, the presence of a ground-glass opacity seems to have positive influence on the prognosis (3). There are also some assumptions that they might be considered as an important parameter in the next clinical T classification. Despite lower incidence of ALK-fusion in lesions with GGO, it is important to routinely test for ALK status as there are also some reports about poorer response to chemotherapy (4). The presented case did not correlate with bulky disease as it may often be expected in patients with canonical ALK-rearrangement partner. So, the correlation between ALK- rearrangement and the radiological features remains still to be investigated. It will be interesting to know whether the patient was offered ALK-TKI and responded in second line.

References

1. Ou, S-H. I. et al. Catalog of 5' Fusion Partners in ALK-positive NSCLC Circa 2020, JTO Clinical and Research Reports, 2020, Volume 1, Issue 1,100015, doi.org/10.1016/j.jtocrr.2020.100015.
2. Yun, J.W. et al. Dysregulation of cancer genes by recurrent intergenic fusions. Genome Biology, 2020, 21:166. doi.org/10.1186/s13059-020-02076-2.
3. Hattori, A et al. Japan Clinical Oncology Group Lung Cancer Surgical Study Group. Prognostic impact of a ground-glass opacity component in clinical stage IA non-small cell lung cancer. J Thorac Cardiovasc Surg. 2021 Apr;161(4):1469-1480. doi: 10.1016/j.jtcvs.2020.01.107.
4. Zhang, Y. et al. Ground-glass opacity-featured lung adenocarcinoma has no response to chemotherapy. J Cancer Res Clin Oncol. 2020 Sep;146(9):2411-2417. doi: 10.1007/s00432-020-03234-6.

Minor remarks/questions

Line 22 – missing space between "NSCLC" and [1]

Changes in the text: Page 2, Line 18.

Line 24 – missing space between "carriers" and [2]

Changes in the text: Page 2, Line 19.

Line 25 – add “radiological” to “subtype”

Besides, it is not established yet that GGO represent a real subtype of NSCLC.

However, it may be used as GGP may be consider as a radiological subtype.

Reply: Thanks for your reminding. To be more precise, we removed “subtype” and indicated these patients with a direct description of “lung cancer with ground-glass opacity (GGO) component”.

Changes in the text: Page 2, Line 21.

Line 27 - missing space between “rapidly” and [3]

Changes in the text: Page 2, Line 22.

Line 27 – delete “genetic”-Otherwise explain the difference between “molecular” and “genetic”

Changes in the text: Page 2, Line 22.

Line 31 - missing space between “progression” and [4]

Changes in the text: Page 2, Line 25.

Line 38/39 - Fig. 1 does not show all the lesions mentioned in the sentence.

Reply: Yes, the figures focused on the primary lesion and the needle biopsy of the enlarged left cervical lymph node was obtained for pathological examination. Right now the imaging of cervical lymph node metastasis was not on hand, hope you can kindly understand.

You can move (Fig.1 A) and rephrase the sentence: “Chest computed tomography scan revealed an irregular -shaped nodule in apicoposterior segment of superior lobe of the left lung (Fig.1 A). Besides nodular ground-glass opacity in inferior lobe of right lung and diffuse bilateral pulmonary nodules”.

Changes in the text: Page 2, Line 30-32. The description was corrected since ground-glass opacity occurred with this irregular -shaped nodule as shown in Figure 1.

Please change the word “apicoposterior” to “localized apical and posterior”

Changes in the text: Page 2, Line 31.

Line 32 - Instead of T1N3M1, it should be T1N3M1b.

Changes in the text: Page 3, Line 37.

Line 46 – which NGS panel was used for detection of this fusion? Mostly RNA-based panels are used for identification of ALK-partner. Were there any co-existing molecular alterations beyond that ALK-fusion?

Reply: Yes, RNA-level validation was widely recommended for fusion detection. In this case, DNA-based NGS panel was used, the genes include EGFR, ALK, RET, ROS1, KRAS, MET, NTRK1/2/3, ERBB2, BRAF. No other alterations were detected with this 9 gene's panel. Due to limited sample, the fusion was only verified with IHC.
Changes in the text: Page 3, Line 40-44.

Line 53 – for to make sure, please explain that the reason for not at choose ALK-TKI in the first line was the price of medicine?

Reply: Yes, the patient knew well about his options. Although ALK-TKI such as crizotinib has been included in reimbursement list, the annual expense might still be higher than chemotherapy (~10 thousands of yuan) according to the price in our hospital and his reimbursement ratio. He finally chose chemotherapy considering his economic condition.

Line 62 - missing space between "therapy" and [2]

Changes in the text: Page 4, Line 60.

Line 68 – remove comma after "that"

Changes in the text: Page 4, Line 66.

Line 70 - missing space between "increasing" and [5]

Changes in the text: Page 4, Line 68.

Line 71 - missing space between "cancer" and [6]

Changes in the text: Page 4, Line 69.

Line 77 - DNA-based NGS is mentioned, please provide the name of the panel.

Changes in the text: Page 3, Line 40-44.

Line 76 – double space between "case" and ","

Changes in the text: Page 4. Line 72.
