Peer Review File

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Reviewer A

Q 1) Authors should mine data (e.g. TCGA) to compare their findings with what exists in the public domain. There are lots of available online platforms to do this. This comparison will improve the external validity of the study, which in the current form is rather simplistic.

A 1) We agree with the suggestion. We made in silico analysis using TCGA database. As a result, unfortunately, high expression of ALKBH4 mRNA in lung adenocarcinoma was not significantly associated with poor prognosis. Many factors may be associated with the discordant result: difference between the TCGA database and our study regarding the criteria for ALKBH4 expression status, oncological profile, patient demographic characters, and anticancer treatment. However, we believe that our results are supported by other study results: according to previous reports on NSCLC, the high expression of ALKBH3 protein in LUAD and high expression of ALKBH5 mRNA are associated with a poor prognosis (ref 11, 12). In addition, we previously clarified by in vitro study that high expression of ALKBH4 in lung cancer cells was associated with increased cell proliferation (ref 14).

Change 1) We added the results and comments regarding in silico analyses in the discussion section (Line 243-254), as follow. According to previous reports on NSCLC, the high expression of ALKBH3 protein in LUAD and high expression of ALKBH5 mRNA are associated with a poor prognosis (11, 12), which may support our current study results. In order to further validate our study, we reviewed the online database Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (32), which is based on the cancer genome atlas (TCGA) and genotype-tissue expression (GTEx) data, containing the gene expression data and survival information of many cancer types. When analysis was performed in 477 patients with LUAD, the overall survival rate in patients with higher ALKBH4 mRNA expression (n=239) was comparable to that in patients with lower ALKBH4 mRNA expression (n=238) (Log-rank test; p=0.57, Hazard ratio = 0.92 for high ALKBH4 expression). The difference between the TCGA database and the current study with regard to the criteria for ALKBH4 expression status, oncological profile, patient demographic characters, and the anticancer treatment could cause the discordant result.

Q 2) Of course, to date, the most important question is if there is a difference in the therapeutic response to particular regimens; prognosis in general is of moderate value.
A 2) We did not investigate whether expression of ALKBH4 would make a difference in the therapeutic response to chemotherapy for NSCLC. Thus, we have mentioned it as limitation of our study.
Change 2) We added the following description in the discussion (Line 281-283).

Q 3) Minor comment: Globally (and in most countries) lung cancer is not the most commonly diagnosed cancer; it is second to breast. It is indeed the highest cause of cancer deaths. Please revise the introduction.
A 3) We agree with the suggestion.
Change 3) We revised the introduction section, as follow: Globally, lung cancer is the second most commonly diagnosed cancer next to the breast cancer and is the highest cause of cancer deaths. (Line 75-76)

Reviewer B
Q 1) This original article study is largely confirmatory of a previously published study by Sci Rep. 2021 Apr 21;11(1):8677.; Cancer Commun (Lond). 2019 Apr 29;39(1):23. Preclinical studies underscore clearly the ALKBH4-E2F1 axis expression was significantly correlated in NSCLC clinical specimens. Patients with high ALKBH4 expression showed a poor prognosis, suggesting that ALKBH4 plays a pivotal tumour-promoting role in NSCLC, and therefore lacks significant novelty of this original study.
A 1) As mentioned in the introduction section (Line 99-107), in our former study (ref 14), the role of ALKBH4 in clinical characters and the prognostic outcome of NSCLC is still poorly understood because the previous study analyzed only selected patients with large adenocarcinoma. In addition, because the previous study only evaluated the results by a univariate analysis, the independent predictive value of ALKBH4 in determining the prognostic outcome remains unclear. In the current study, we comprehensively evaluated patients with NSCLC without any selection criteria regarding tumor size and histologic subtypes by univariate and multivariate analyses. We also reviewed previous reports and considered the role of the ALKBH family expression in various cancers. As a result, ALKBH4 expression was associated with poor prognosis in patients with lung adenocarcinoma, but not in patients with lung squamous cell carcinoma. In addition, ALKBH4 expression revealed to be an independent prognostic factor for relapse free survival and overall survival based on multivariable analysis.

Change 1) Not changed. We revised our manuscript in response to the reviewer A's suggestion.