Peer Review File

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Comment 1： Despite this, a key point is that the manuscript lacks novelty, since it is very similar to the study published by Xu et al in Oncology Letters in 2018 (doi: 10.3892/ol.2018.9044). In this work by Xu et al, authors assessed the prognostic role and biological function of IL-6R mRNA expression in LUAD and LUSC. In that study, authors also used the TCGA cohort. Findings are also similar, showing that lower levels of IL-6R were associated to lower survival in LUAD, but not in LUSC. Authors also showed that IL-6R and its correlated genes were primarily involved in cell cycle progression in LUAD, and in tumor angiogenesis, invasion and metastasis in LUSC. In this manuscript, authors did not cite neither discuss the study by Xu et al, which is fundamental in the context. Secondary findings presented in this manuscript by Sun et al (e.g., TNM as a risk factor for Lung Adenocarcinoma) are also well known from the literature.

Reply 1： We are grateful to reviewer for his/her effort reviewing our paper and his/her positive feedback. According to the review comments put forward by the editor, what we want to say is that this research does have some similarities with the previous research of Xu et al., but the main concern of this research is still very different from that of Xu et al. First, Xu et al.'s study only studied the prognostic survival analyses of IL6R for LUAD and lung squamous cell carcinoma through the TCGA database and GEO database. The purpose of this study was to explore the value of IL6R in predicting the survival rate of patients with LUAD at different stage, and to study the prevention of overtreatment in patients with LUAD. Here, we explored the correlation between IL6R and the prognosis of LUAD patients of different age, gender, smoking status, and TNM stage through subgroup analyses. In addition, the prognostic value of IL6R in patients with LUAD was analyzed, and the prognostic nomogram was established by integrating IL6R with other risk factors that affected the prognosis of patients with LUAD. Finally, this study also analyzed IL6R-related immune responses and signaling

pathways through GO, KEGG, and GSEA. These were not involved in the research of Xu et al.

Changes in the text： We have modified our text as advised (see page 5, line 94-104).

Comment 2： Thus, the main novelty of the current manuscript consists in a general description of the relation between IL-6R expression and immune cells. However, these analyses are quite superficial, as well as the description of results. With this in mind, I believe that several data related to the prognosis role of IL-6R (Figure 1 to 3) should be transferred to supplementary Figures, since results are very similar to that from Xu et al. I suggest that authors explore better the NTU cohort. In parallel, authors need further explore the relation between IL-6R and immune system in a translational manner. For instance, authors should assess the impact of key markers of immune system in survival analysis leading into consideration levels of IL-6R.

Reply 2： Further explore the relationship between IL-6R and the immune system based on the reviewer's proposal. For example, to evaluate the impact of key immune system markers in survival analyses to take into account the level of IL6R. We conducted statistical analysis and found that among the 11 immune cells associated with IL6R found in the previous analyses of this study, M0 macrophages cells and resting dendritic were significantly associated with the 5-year overall survival of patients with LUAD. The remaining immune cells had no significant correlation with the prognosis of LUAD. Among them, the expression level of M0 macrophages was negatively correlated with the 5-year overall prognosis of patients with LUAD (P=0.012; Fig. 6A), while resting dendritic cells was positively correlated with the 5-year overall prognosis of patients with LUAD (P=0.043; Fig. 6B). This has been clarified in the revised version of the manuscript, and Figure 6A and 6B has been included in the revised manuscript as new Figure.

Changes in the text： We have modified our text as advised (see page 12, line 241-250).

Comment 3： The organization of results is quite confusing. Figure 4, 5 and 6 explore the connection between IL-6R and immune system cells, while Figure 1, 2, 3 and 7 are related to prognosis and survival. I suggest reorganizing this and separating each goal (i.e., firstly all results from prognosis, and then all results related to immune system).

Reply 3： This suggestion is appreciated. We have changed the positions of Figure 4, 5 and 6 with Figure 7.

Changes in the text： We have modified our text as advised (see page 10-12, line 206-214, 215-237).

Comment 4： Considering P.11 l.218 (results from Figure 4) – Actually, each immune cell may express specific levels of IL-6R. With this in mind, the results do not support the conclusion that these populations of cells 'were affected by the expression of IL-6R'. For instance, another possibility is that the different profiles of immune cells may impact the levels of IL6R. This should be better discussed in the manuscript, including the presentation of the levels of IL-6R expression in each of these subpopulations of immune cells presented in Figure 4A.

Reply 4： According to the question raised by the reviewer, we have checked many literatures and found that there are few related studies on how IL6R interacts with related immune cells. This article only studies the relationship between IL6R and related immune cell statistics, and has not yet explored how IL6R and related immune cells affect each other. This is also the direction of our future research.

Comment 5： The authors should include a supplementary image with some immunohistochemistry samples from NTU cohort (as in Fig. 1A). If possible, authors may include paired samples for the same patient (normal x tumor sample). If possible, authors may include a comparative quantification of IL-6R between tumor and normal samples from this cohort.

Reply 5： According to the reviewer’s suggestion, we have selected some typical pictures of IHC staining of cancer and para-cancerous tissues and placed them in Supplementary Figure 1. However, we are very sorry that due to conditions, we have

not been able to obtain the tissue microarray of the matched sample of the same patient. We also failed to perform a quantitative comparison of IL-6R between tumor samples and normal samples.

Comment 6： Authors could analyze the Kaplan-Meier curves combining IL-6R levels and the genetic status (i.e. whether mutated or not) or expression levels of driver genes in lung cancer (e.g., EGFR, KRAS, etc.)

Reply 6： We thank the reviewer for this comment. According to the reviewer's recommendations, we analyzed the differential expression of EGFR and KRAS genes in LUAD patients with high and low IL6R expression groups and analyzed the effects of EGFR and KRAS genes on the OS of LUAD patients with high and low IL6R expression groups. The results showed that the expression of EGFR gene in LUAD patients with low IL6R expression was significantly higher than that in LUAD patients with high IL6R expression ($P<0.001$) (Fig. 8A). However, no significant difference in KRAS gene expression was observed in patients with LUAD with high and low IL6R expression (Fig. 8B). Then combined with the expression level of IL6R and the Kaplan-Meier curve of the expression levels of lung cancer driver genes EGFR and KRAS, the results showed that EGFR and KRAS genes had no significant difference in the survival of LUAD patients between the IL6R high and low expression groups (Fig. 8C and 8D). This has been clarified in the revised version of the manuscript, and Figure 8A-8D has been included in the revised manuscript as new Figure.

Changes in the text： We have modified our text as advised (see page 13, line 257-270).

Comment 7： Several figures are poorly described in the results section. Similarly, several legends are poor (mainly from Figure 5). The lack of information in the legend added to the poor description of the results practically precludes the critical interpretation of some figures.

Reply 7： We have carefully addressed the reviewer's concern. Based on the reviewer's suggestions, we have re-described the results in Figure 7 fully. We divided LUAD

patients into two different groups (high and low immune infiltration groups) according to the level of immune cell infiltration. Then analyzed the relationship between different immune infiltration groups and IL6R expression levels and other clinical parameters. Finally, we learned that the expression of IL6R may be positively correlated with immune cells infiltrating tumors. The more tumor immune cells infiltrated, the earlier the patient's clinical stage, and the higher the 5-year overall survival rate. The result was shown in Figure 7.

Changes in the text： We have modified our text as advised (see page 12-13, line 251-256).

Minor point 1： Authors must make clear the sample number used in the survival curves (include in the legend).

Reply 1： As requested we have make clear the sample number used in the survival curves (include in the legend).

Changes in the text： We have modified our text as advised (see page 18, line 375-377).

Minor point 2： What is the difference between Fig. 1B and 1C? Please, make it clearer.

Reply 2： Figure 1B is a comparison of IL6R mRNA expression levels in 500 LUAD samples and 21 paracancerous samples in the TCGA cohort. Figure 1C is a comparison of IL6R mRNA expression levels in paired samples in the TCGA cohort.

Changes in the text： We have modified our text as advised (see page 8, line 161-165).

Minor point 3： Fig. 3A and B – change the ordering of variables (IL-6R should be the first variable in the graph).

Reply 3： We have changed IL6R to the first variable in the chart based on the reviewer's suggestion.

Minor point 4： Results from Fig 4 are quite descriptive and confuse. I suggest that authors rewrite these sentences more objectively.

Reply 4： We have placed Figure 4 in the position of Figure 5. We have described Figure 5 more objectively based on the reviewers' suggestions.

Changes in the text： We have modified our text as advised (see page 11-12, line 225-237).

Minor point 5： Fig 4B should be supplementary, since it is not related to IL-6R.

Reply 5： We have put Figure 4B in the figure supplementary 2 based on the reviewer's recommendation.

Minor point 6： In Figure 5, what do clusters mean? Authors should make this clearer. Authors may also increase the font size in Figure 5.

Reply 6： Cluster means high and low immune infiltration group.

Changes in the text： We have modified our text as advised (see page 12, line 251).

Minor point 7： In the following sentence (P.11 l.213) – "The CIBERSORT algorithm applied to 21 immune cell subtypes helped to evaluate the difference in expression levels between high and low IL-6R expression groups (Fig. 4A)". Actually, it is not 'the difference in expression levels' between high and low, but the enrichment of different subpopulations of immune cells, isn't it?

Reply 7： Yes，it is the enrichment of different subpopulations of immune cells.

Changes in the text： We have modified our text as advised (see page 11, line 224).

Minor point 8： Authors may review and rewrite the following sentences:

- P.4 l.45 – "low diagnosis rate in the early stage of the disease"
- P.4 l.81 – "polymorphisms were associated with the reduction of lung cancer damage (?)"

Reply 8： We have rewritten the two sentences pointed out by the reviewer.

Changes in the text： We have modified our text as advised (see page 4, line 67),（see page 4, 82-83）.

Minor point 9： The language is poor and full of typographic mistakes. I strongly suggest that authors submit the manuscript to an English editorial office.

Reply 9： We have sent our manuscript to the AME editorial department for language editing. The manuscript has been polished up. English Language Editors: J. Gray and C. Betlazar-Maseh

Minor point 10： Some words in the introduction are capitalized.

Reply 10： We have capitalized some words in the introduction.

Changes in the text：We have modified our text as advised (see page 2, line 37),（see page 3, line 44-45, 48, 51-53, 55）.

Minor point 11： Authors should use the abbreviation, not the full name, from the first time the abbreviation appears in the text.

Reply 11： We have followed the reviewer's recommendation to use the abbreviation instead of the full name from the first time it appears in the text.

Changes in the text： We have modified our text as advised (see page 4, line 61, 63,84),（see page 5, line 98), .(see page 6, line 107-110, 114, 122),（see page 7, line 133, 140),. (see page 8, line 165),（see page 9, line 170, 179, 181), .(see page 10, line 193, 195),（see page 11, line 212, 213),.(see page 14, line 284),（see page 15, line 303-305),.(see page 16, line 318-320,332,335-336),（see page 17, line 338),.(see page 18, line 374), (see page 19, line 378,382,385,388).