Peer Review File

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Reviewer A

In this manuscript, Huang et al. report a new potential mechanism that underlies the different bleeding outcomes in LUDA and LUSC patients after bevacizumab treatment. The authors initially confirmed that LUAD tumour samples, which cause less bleeding cases, contain higher MVD that that of LUSC tumours samples. In vivo and in vitro experiments confirm that LUAD cell line is more capable of promoting angiogenesis, nevertheless their VEGF expression and secretion are comparable. By exploring the single-cell sequence data, the authors find that genes IRF7 and IFIT7 are upregulated in LUAD cells and LUSC cells respectively. Finally, they investigate the different role of these genes in angiogenesis, and show that bevacizumab can promote their roles on angiogenesis by differently regulation their expression. This work is quite interesting; however, some issues muse be addressed before publication.

1. Were IRF7 and IFIT7 regulated by Bevacizumab or different tumour cell cocultured with them. This is another important issue to be discussed.
Reply1: Thank you for the reviewer's helpful comment, in Figure5, we verified that *IRF7* and *IFIT2* expression in endothelial cells would change after it co-cultured with lung cancer cells. As verified in Figure 7, the fold difference in *IRF7* and *IFIT2* expression was greater after the co-cultured endothelial cells added bevacizumab. This suggests that bevacizumab can further regulate the expression of these two genes.

2. What's the relationship between these two genes, are they connected in a same pathway?
Reply 2: Thank you for pointing this out, both genes are interferon regulatory genes(1,2), but no pathway has been found that coexists with these two genes at present.

3. The authors should briefly introduce the known functions of IRF7 and IFIT7 in results part rather than only in discussion.
Reply 3: Thank you for the reviewer's helpful comment, we added some known functions of *IRF7* and *IFIT2* in results part as advised.
Changes in the text: page 11, line 345-347.

4. How long did these patients have bevacizumab treatment, did they get same duration of treatment? This information should be included in Table 1.
Reply 4: Thank you for pointing this out, at past, in the treatment of NSCLC patients with bevacizumab, Sandler et al proposed "In the phase 2 study that served as the impetus for our trial, an unexpectedly high rate of life-threatening and fatal pulmonary hemorrhages was associated with bevacizumab treatment, particularly in patients with squamous-cell lung cancer".(3) To further explore the mechanism of this phenomenon, we collected 36 cases of

NSCLC tissue for IHC analysis of microvessel density (MVD) in the two different tumors. Due to its huge side effects, bevacizumab is not recommended for patients with LUSC in the current clinical guidelines. Therefore, the current clinical cases of LUSC have no history of bevacizumab treatment.

5. The full name of cell line should be properly listed in Method section and figure legends. Quite confused while reading figures.

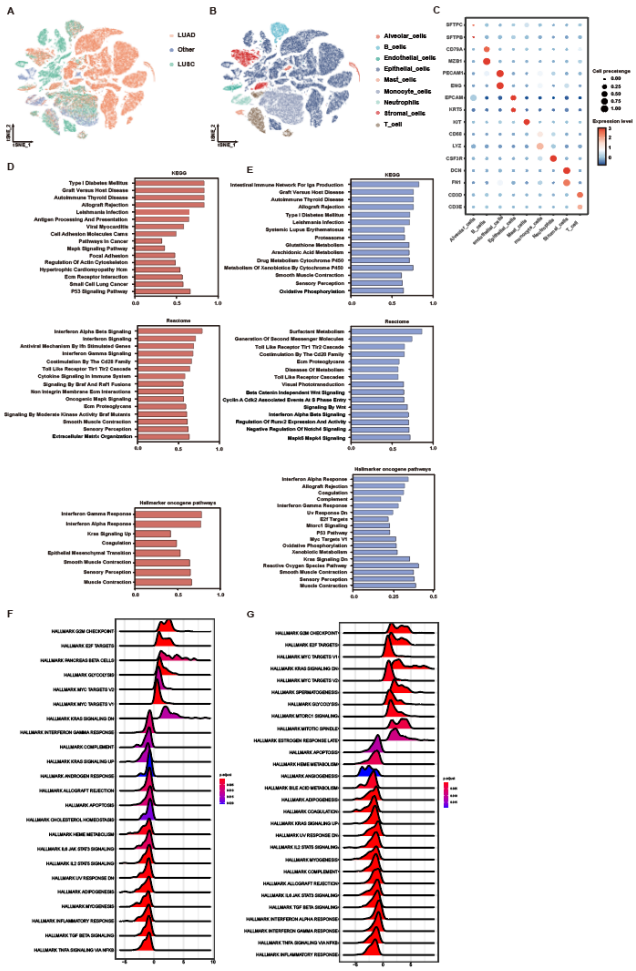
Reply 5: Thank you for the reviewer's helpful comment, we complemented the full name of cell line as advised.

Changes in the text: page 7, line 198; page 7, line 206; page 9, line 295; page 10, line 308; page 12, line 369; page 19, line 569; page 22, line 603; page 24, line 625.

Reviewer B

In the study, the authors have identified two target genes (IRF7 and IFIT2) that may play an important role in the angiogenesis of LUAD and LUSC, and account for different hemorrhage outcomes caused by bevacizumab. The work is interesting, however presented several minor flaws, that can be addressed.

1, For the bioinformatic analysis of figure 4, it is desirable that the authors include an analysis of the difference in signal pathways between LUAD and LUSC.



Reply1: We agree with the reviewer's comments. We complemented an analysis of the difference in signal pathways between LUAD and LUSC as advised.
Changes in the text: page 22, line 590.

2, There is no correlation analysis between two gene expression levels and MVD in lung cancer tissue, how can authors make a conclusion that "Higher IRF7 levels and lower IFIT2 levels in LUAD tumors were associated with a higher MVD in LUAD tissues"?

Reply2: Thank you for the reviewer's helpful comment, through immunohistochemistry, we found that LUAD had more microvascular density. Two differentially expressed genes, *IRF7* and *IFIT2*, were screened out by combining the single cell sequencing data of NSCLC with experimental verification. At the same time, the two genes were knocked down to verify their influence on angiogenesis. It was found that high level of *IRF7* and low level of *IFIT2* were more conducive to promoting the formation of microvessels.

3, What is the change in two genes' expression before and after adding bevacizumab?

Reply3: Thank you for pointing this out, we found differential expression of *IRF7* and *IFIT2* in *LUSC* and *LUAD* patients tissue through bioanalysis. In Figure 5, we verified that *IRF7* expression in endothelial cells was up-regulated by 4-6 times after co-culture with LUAD cells, and *IFIT2* expression was up-regulated by 3-5 times after co-culture with LUSC cells. In Figure

7, after adding bevacizumab, *IRF7* and *IFIT2* expression were upregulated by 13-fold and IFIT2 expression in endothelial cells co-cultured with tumor cells, which proved that the difference between the above two genes increased after the addition of bevacizumab.

4, This study was not carried out in the samples of patients who were treated with bevacizumab, and it is recommended to remove the description of "patients 43 with non-small cell lung cancer after bevacizumab treatment".
Reply4: Thank you for the reviewer's helpful comment. We did not fully consider it in the writing process.
Changes in the text: we have modified our text as advised deleted "after bevacizumab treatment". (see page 2, line 43)

1. Martire S, Navone ND, Montarolo F, et al. A gene expression study denies the ability of 25 candidate biomarkers to predict the interferon-beta treatment response in multiple sclerosis patients. J Neuroimmunol 2016;292:34-9.
2. Yi F, Hu J, Zhu X, et al. Transcriptional Profiling of Human Peripheral Blood Mononuclear Cells Stimulated by Mycobacterium tuberculosis PPE57 Identifies Characteristic Genes Associated With Type I Interferon Signaling. Front Cell Infect Microbiol 2021;11:716809.
3. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355:2542-50.

Reviewer C

The author evaluated the differential expression of microvascular density (MVD) in tumor tissue from LUAD and LUSC patients, and then downloaded and analyzed single-cell sequencing data obtained from cancer tissue to identify differentially expressed genes related to angiogenesis. The results showed that the MVD of LUAD tissue was higher than that of LUSC tissue. Further evidence suggests that higher levels of IRF7 and lower levels of IFIT2 in LUAD tumors are associated with higher MVD in LUAD tissues.
The study is novel and practical, which to some extent reveals that IRF7 and IFIT2 may be related to bleeding after bevacizumab treatment. But it is far from finding new mechanisms, and needs more in-depth research. It might need to revise the accuracy and objectivity of wording.
Reply: Thank you for the reviewer's helpful comment. We did not fully consider it in the writing process, we have made the following modifications.
Changes in the text: We have modified our text as advised. (see Page 2, line 56; Page 2, line 64; Page 3, line 139; Page 12, line 390)

Reviewer D

1. Figure 1
Please provide the staining method of Figure 1A and 1B in the legend.
Reply: modified.

2. Figure 2

a) Please provide the observational method of Figure 2A in the legend.

b) Please explain ns and MVD in the legend.

Reply: modified.

3. Figure 3

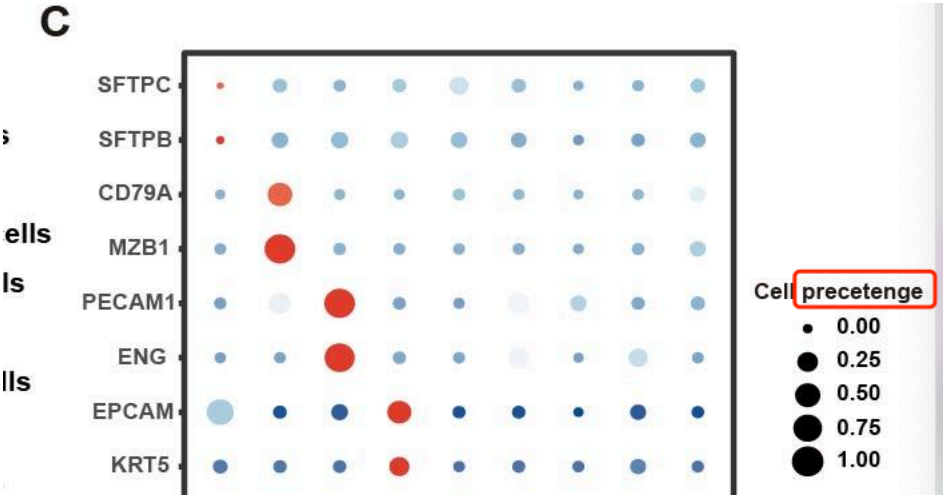
Please explain DAPI, ELISA, LUSC and NS in the legend.

Reply: modified.

4. Figure 4

a) Please explain LUAD, LUSC, t-SNE, and KEGG in the legend.

b) Please check if the word is correct.



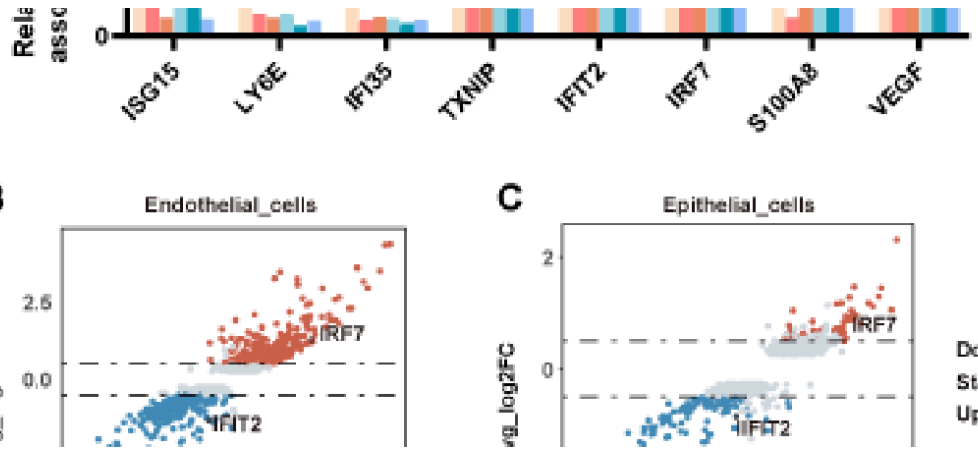
c) Where's the top bars and number in brackets in figure? Please check.

633 marker genes in the indicated cell types. The top bars indicate the clusters
634 corresponding to specific cell types, and the number in brackets corresponds to the
635 cluster number. (D and E) Bar chart showing the enrichment of specific pathways based

Reply: modified.

5. Figure 5

a) Please provide a clearer version of figure 5, the current one cannot be seen clearly (as you can see the screenshot below).



b) Please explain the meaning of * and ** in the legend.

c) Please explain FC in the legend.

Reply: modified.

6. Table 1

Please unify the format.

Age(years)	62.9±7.9

Data are expressed as number of patients, mean (SD) or percentage. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SD, standard deviation.

Reply: modified.

7. References/Citations

a) There are 2 reference lists in the file, please keep the correct one and delete another one.

b) Please check if the author's name matches with the citation.

410 endothelial cells of LUSC tumors. Karin et al. reported that IRF3 and *IRF7* mediate
411 neoangiogenesis through inflammatory cytokines (28). Lai et al. showed that *IFIT2*

c) Please add the citation for Eric et al. at the end of the sentence.

427 It is generally believed that MVD is positively correlated with the risk of bleeding. Eric
428 et al. collected the clinical data of 222 patients with NSCLC treated with bevacizumab

d) Please double-check if citations should be added as you mentioned "studies".

394 lung cancer. Several studies have shown that bevacizumab-based therapy may lead to
395 longer survival and significantly prolonged disease remission in patients with advanced
396 NSCLC. However, patients with LUSC are prone to massive hemoptysis during

Reply: modified.