

Peer Review File

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Comment 1 from Reviewer 1. Tumor microenvironment plays an indispensable role in the occurrence, development and metastasis of tumors, and affects the therapeutic effect of tumors. How to evaluate the immune status of TME and judge the prognosis of lung SCC? It is recommended to add relevant content.

Response to comment 1. We agree with the reviewer's suggestion. Determining the role of the tumor immune microenvironment (TIME) in lung SCC may reveal the immune mechanisms mediating disease progression and may facilitate the identification of prognostic biomarkers. In the current study, we attempted to examine the associations of CD3/CD8, which may predict cancer outcomes, with CAF-related protein patterns in lung SCC. Here, although we classified lung SCC into two subgroups using cluster analysis, the CD3/CD8 ratio, which has been shown to be correlated with patient prognosis in several cancers, including colorectal cancer, hepatocellular carcinoma, and urothelial carcinoma, was not correlated with subgroups classified according to prognosis in patients with lung SCC (subgroup 1 > subgroup 2). In addition, the CD3/CD8 ratio was also not correlated with prognosis in patients with

lung SCC (not retained in univariate analysis). From this finding, we suggest that the expression pattern of CAF-related proteins secreted from CAFs may be independent of the CD3/CD8 ratio (**Supplementary Methods**; page 3, line 15-17; page 4, line 5; page 17, lines 17–page 18, line 3; page 23, lines 15–page 24, line 4).

Comment 2 from Reviewer 1. *The figures in this study have many obvious errors. The order of Figures 2 and 4 is reversed, Figure 5 does not appear in the manuscript and in the legend. Supplementary Figures 1 and 2 are only have legends and no figures.*

Please check carefully and make corrections.

Response to comment 2. We agree with the reviewer’s suggestion. We have changed the order of the figures according to the reviewer’s comment.

Comment 3 from Reviewer 1. *The REMARK checklist is not filled out, please complete it.*

Response to comment 3. We appreciate this comment from the reviewer. We have checked the *REMARK checklist* again in accordance with the reviewer’s suggestion.

Comment 4 from Reviewer 1. *What are the characteristics of different CAF*

classifications? What role do they each play in lung SCC? It is recommended to add relevant content.

Response to comment 4. We agree with the reviewer's comment. Our aim was to identify the expression patterns of CAF-related markers in lung SCC without arbitrariness. As a result, we could classify lung SCCs into two subgroups (subgroup 1 and 2). In addition, we found that patients in subgroup 1 had a worse prognosis than patients in subgroup 2. This finding suggested that the expression pattern of CAF-related markers could contribute to evaluation of prognosis in patients with SCC.

Among the CAF-related markers examined in this study, we found that AEBP1, CD10, FAP, PDGFR β , FSP1, TWIST1, and ZEB1 expression levels in CAFs were significantly higher in subgroup 1 than in subgroup 2. Furthermore, we found that only AEBP1 expression was correlated with a worse prognosis in patients with lung SCC.

These findings were already described in the text (page 18, line 15 to page 19, line 2; page 19, line 3 to line 14).

Comment 5 from Reviewer 1. *There have been many studies on lung SCC and CAF.*

What is the difference between this study and previous studies? What is the innovation?

These need to be described in the introduction.

Response to comment 5. We agree with the reviewer's comment. The difference between the current study and previous studies was that we aimed to identify heterogeneous expression of CAF-related proteins in lung SCC. In addition, such expression patterns (here, subgroups 1 and 2) affected prognosis in patients with lung SCC. This finding suggested that the specific CF-phenotype (subgroup) was closely associated with worse prognosis in patients with lung SCC. This study is the first to identify the association of the heterogeneous expression patterns of CAF-related proteins (the specific CAF-phenotype), which could be stratified according to cluster analysis, with outcomes in patients with lung SCC (page 6, lines 16 to page 7, line 2).

Comment 6 from Reviewer 1. *How to use bioinformatics to mine the core genes of lung SCC and analyze the survival prognosis of patients? It is recommended to add the content of the discussion.*

Response to comment 6. We agree with the reviewer's suggestion. In the current study, we examined the associations of known CAF-related markers (antibodies) with

outcomes in patients with lung SCC given that antibodies must show reliability and reproducibility when used to evaluate the expression patterns of CAF-related markers. However, for the discovery of new CAF-related markers, genome-wide analysis may contribute to comprehensive evaluation of mRNA expression occurring in cancer stromal tissue. In our experience, isolation of the surrounding stromal cells containing CAFs may enable identification of mRNAs that are closely associated with prognosis in patients with lung SCC. However, despite great efforts, the discovery of such new CAF-related markers is actually very difficult, even when using genome-wide analysis. Further studies are needed in the near future (page 24, lines 13–page 25, line 2).

Comment 7 from Reviewer 1. *What is the mechanism by which CAF regulate tumor drug resistance? It is recommended to add relevant content.*

Response to comment 7. We agree with the reviewer’s suggestion. We have added the following text to the revised manuscript:

“CAF-induced resistance to chemotherapy and radiotherapy in lung cancer is closely associated with several factors, including cytokines, chemokines, growth factors, and exosomes. The molecular mechanisms mediating resistance to chemotherapy and radiotherapy have been evaluated. Cytokines and chemokines are inflammatory

mediators secreted by cancer cells or CAFs in the TME and can stimulate tumor-promoting processes, including proliferation, metastasis, and progression, in an autocrine or paracrine manner. In addition, the cytokines and chemokines in the TME are strongly related to chemoresistance and poor prognosis in patients with cancer. In lung adenocarcinoma cells in vivo and in vitro, IL-11 was found to be able to protect cancer cells from cisplatin-induced apoptosis and thus promote their chemoresistance. As a result, CAFs treated with cisplatin confer chemoresistance to lung cancer cells. Furthermore, cancer-secreted TGF- β can enhance the transition of resident fibroblasts into CAFs, and CAF-secreted TGF- β is involved in cancer therapy resistance in cancer cells. Finally, various studies have examined the roles of exosomes in cancer progression. The function of CAF-derived exosomes in cancer therapy resistance was initially investigated in CRC. Hu et al. reported that CAF-derived exosomes promote drug resistance by mediating the activation of the Wnt signaling pathway in CSCs in CRC. Understanding the molecular mechanisms mediating chemoresistance by CAFs will become even more important in this field.” The paragraph has been added to the revised Discussion section (page 22, line 13 to page 23, line 14).

Comment 8 from Reviewer 1. *It is recommended to add research progress on the relationship between CAF and lung SCC to the discussion.*

Response to comment 8. We agree with the reviewer's suggestion. This study provides a better understanding of the actual roles of CAF-related proteins in determination of outcomes in patients with lung SCC because it focuses on the functional aspects of CAFs surrounding cancer cells in the invasive area and takes into account that the expression of specific CAF-related proteins plays important roles in cancer progression via the formation of the TME. In the current study, we found that high AEBP1 expression was helpful for predicting patient prognosis and may characterize CAF classification. In addition, the current results may facilitate the discovery of key therapeutic targets for new drug development. This information has been incorporated into the revised manuscript according to the reviewer's suggestion (page 26, lines 1–5).