

Peer Review File

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Reviewer A

The authors present very compelling data. I have a few comments:

1. The title is not accurate this study reports results only for SCC and the title should reflect that. There is no data about AC, the title is ambiguous in that sense.

Reply: This is an excellent point, and we appreciate the thoughtful evaluation of our work. Given the study just reported the results only for SCC, we are in complete agreement that we need to modify the title of this manuscript, and we have revised “Esophageal cancer” to “esophageal squamous cell carcinoma” in the title. In addition, we have also corrected “EC” to “ESCC” through the entire manuscript where corrections were needed.

2. There is not clarity what the authors mean with "non surgical" EC. Words like locally advanced are used and the patients have a variety of stages from I-V meaning that many of those patients could have been surgical after neoadjuvant therapy, and for stage I patients even without therapy. Therefore is important to clarify why those were not surgical either patient had metastatic or unresectable disease (stage IV) or patients were to infirm to have surgery. This will help understanding the data, because is har to call locally advance disease for stage I.

Reply: We are very sorry for any confusions caused to you and We indeed made some mistakes. We have rechecked the TNM stages of all the patients enrolled in this study, and found that we only have patients with stage III and IV esophageal squamous cell carcinoma (ESCC) . However, as shown in Table 1 and the

"Description of patient cohort" of Results section, we had written stages I-III, and it was indeed a low-level mistake. The fact was that the study included one patient with stage IIIA, 12 patients with stage IIIB, and 30 patients with stage IV. For this stage IIIA patient, due to his old age (81 years) and severe hypertension, it has been decided not to perform surgery after full communication with the families. For patients with stage IIIB, some patients have tumors located in the upper part of the esophagus, and the tumor position was relatively high; some patients had a higher number of positive lymph nodes, and some of the lymph nodes are located quite far from the tumor site; some patients had tumors located in the lower part of the chest, and experienced clavicular lymph node metastasis. The potential benefits of surgry for these patients may be somewhat controversial, and after multidisciplinary discussions and full communication with the patients' families, it was decided not to proceed with surgery for these patients. Instead, other treatment options such as radiotherapy has been considered.

In the revised manuscript, we have corrected the description of Stage “I-III” to “IIIA” and “IIIB” in Table 1 and the “Description of patient cohort” of Results section. Besides, considering that we enrolled stage III patients in this study, we decided to modify the Title “Molecular characteristics and multivariate survival analysis of 43 patients with advanced esophageal cancer” to “Molecular characteristics and multivariate survival analysis of 43 patients with locally advanced or metastatic esophageal squamous cell carcinoma”

We deeply apologize once again for the mistake we made, which has caused confusion for both you and ourselves. We hope that you will understand and forgive us.

3. Same in the discussion focusing on the data and comparison with SCC is important but generalization about AC should not be attempted.

Reply: We appreciate the thoughtful evaluation of our work and we are in complete agreement with your suggestion. We have already modified the descriptions of “EC” to “ESCC” of our own ESCC cohort. The major modifications are as follows:

- Page 9, Line 183-184

Original: In this study, we analyzed the molecular characteristics of 43 EC patients in China using a pan-carcinomatous NGS package containing 425 genes.

Revised: In this study, we analyzed the molecular characteristics of 43 **ESCC** patients in China using a pan-carcinomatous NGS panel containing 425 genes.

- Page 11, line 219-225

Original: In this study, we conducted a correlation analysis between abnormalities in NOTCH signaling pathway and OS in esophageal cancer patients, and found that the alterations in NOTCH signaling pathway had poor OS in esophageal cancer patients, suggesting that abnormal NOTCH signaling pathway are related to the development of esophageal cancer and may have an intrinsic tumorigenic effect, which is consistent with previous reports. However, due to different research methods and small sample size, the complexity of NOTCH1 signaling pathway in esophageal cancer still needs to be explored for a long time.

Revised: In this study, we conducted a correlation analysis between abnormalities in NOTCH signaling pathway and OS in **ESCC** patients, and found that the alterations in NOTCH signaling pathway had poor OS in **ESCC** patients, suggesting that abnormal NOTCH signaling pathway are related to the development of **ESCC** and may have an intrinsic tumorigenic effect, which is consistent with previous reports. However, due to different research methods and small sample size, the complexity of NOTCH1 signaling pathway in **ESCC** still needs to be explored for a long time.

- Page 12, line 238-240

Original: In this study, univariate and multivariate Cox regression analysis showed that CBLB mutation was associated with poor prognosis in patients with

esophageal cancer.

Revised: In this study, univariate and multivariate Cox regression analysis showed that CBLB mutation was associated with poor prognosis in patients with **ESCC**.

- Page 12, line 250-251

Original: In this study, TSC2 mutation was significantly associated with poor OS in esophageal cancer patients.

Revised: In this study, TSC2 mutation was significantly associated with poor OS in **ESCC** patients.

- Page 13, line 267-269

Original: Nonetheless, this result could offer a more comprehensive perspective for our subsequent research on TMB in predicting the immune efficacy of esophageal cancer, not just confined to high TMB.

Revised: Nonetheless, this result could offer a more comprehensive perspective for our subsequent research on TMB in predicting the immune efficacy of **ESCC**, not just confined to high TMB.

Reviewer B

1. Please provide a summarized legend for Figure 3,4,5, followed by legends for each part.

Reply: Summarized legends have been added.

2. The citation of Figure 4A is missing in the main text.

Reply: The citation of Figure 4A in the main text has been added.

3. All abbreviations in figures/tables and legends should be explained.

Reply: Revised as requested.