Peer Review File

Article information: https://dx.doi.org/10.21037/atm-21-4015

Replies to Reviewer A

Comment 1: COX2 and SOX2 are reliable markers for esophageal cancer, but please describe the difference from the above markers in terms of sensitivity

Reply 1: As the reviewer mentioned, COX-2 and SOX2 have been widely recognized as reliable markers for esophageal adenocarcinoma. Their role in the initiation and progression of esophageal adenocarcinoma has been relatively clear. For example, neoplastic progression of BE towards EAC is highly associated with increased expression of COX-2 (Reference 54). Selective COX-2 inhibition down-regulates COX-2 and MET expression, both important molecules involved in EAC progression and dissemination (Reference 55). While SOX2 expression is lost during transition from BE to EAC, and is related to an increased risk of neoplastic progression (Reference 57). In addition, pattern of p53 and particularly SOX2 protein expression in EAC predicts response to neoadjuvant chemoradiotherapy (Reference 58). Compare with above 2 biomarkers, these identified through our bioinformatic analysis seems less credible due to lack of functional experiments validation. Therefore, the expression, molecular mechanism and prognostic role of the potential biomarkers warrant further experimental studies to elucidate, which might be a focus in future research. In order to make this point more clear, we have further explained it in the discussion part. (see Page 18, line 376-391)

Change in the text: We have added a paragraph to the text in discussion part writing “It is widely recognized that cyclooxygenase-2 (COX-2) and SRY-box transcription factor 2 (SOX2) are reliable biomarkers for EAC. COX-2 plays important roles in the induction of inflammation and tumorigenesis (53), and neoplastic progression of BE towards EAC is highly associated with increased expression of COX-2.(54) Selective COX-2 inhibition downregulates COX-2 and MET proto-oncogene (MET) expression, which are both important molecules involved in EAC progression and dissemination.(55) SOX2 is a transcription factor associated with cancer stem cells (CSCs) and embryonic stem cells, and is involved in the formation and differentiation of esophageal epithelium.(56) SOX2 expression is lost during transition from BE to EAC, which is related to an increased risk of neoplastic progression.(57) In addition, the pattern of p53 and particularly SOX2 protein expression in EAC predicts the response to neoadjuvant chemoradiotherapy (nCRT).(58) Compared with the above 2 biomarkers, those identified through our bioinformatic analysis seem less credible due to the lack of functional experiments. Therefore, further experimental studies to elucidate the expression, molecular mechanism, and prognostic role of the potential biomarkers are required.” (see Page 18, line 376-391)

Comment 2: There seems to be a difference in molecular biology between SCC and

ADC. Author write the above biomarker in ADC. Please comment the above biomarker in SCC.

Reply 2: EAC and ESCC are the two main pathological types of esophageal cancer. Biologically, ESCC shares many characteristics with squamous cell carcinoma of the head and neck, whereas EAC resembles chromosomally unstable gastric adenocarcinoma in its genetic makeup. We have already discussed the biomarkers identified through our analysis in EAC. Actually, some of the above biomarkers have been verified in ESCC. For example, a recent study has demonstrated that high expression of BGN in ESCC tumor sample was validated by RT-qPCR, suggesting BGN might associated with a poor prognosis. It has been verified that miR-133a-3p inhibited the cell propagation, invasion, and migration and facilitated apoptosis in ESCC by targeting COL1A1. Methylation of CLDN3 is common in the esophageal mucosa of patients with ESCC in this high-risk population, and tends to increase in prevalence in foci with increasing histological severity of disease. In addition, PLAU was not only a prognostic marker of ESCC, which promoted tumor cell proliferation and migration, but also promoted the formation of inflammatory cancer-associated fibroblasts (CAFs) by the PLAU secreted by tumor cells. However, since our research only focuses on esophageal adenocarcinoma, we have not discussed the role of the above biomarkers in esophageal squamous cell carcinoma in this article.

Change in the text: No change.

Special thanks to you for your good comments.

Replies to Reviewer B

Comment 1: Although an ethical statement is made, I think this is insufficient. Was this study approved by any local board? Who approved use of data? Was data anonymized? Did patients in the test group signed informed consent?

Reply 1: We are very sorry for our negligence of clarifying this problem in a more detailed way. Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Change in the text: No change.

Comment 2: Figure 8: Kaplan meier curves are really small.

Reply 2: We are very sorry for providing relative small Kaplan-Meier curves (Figure 8) in our manuscript. We have remade it to meet the requirements of the reviewer. The corrected figure 8 has been resubmitted with the revised manuscript.

Change in the text: No change.

Comment 3: Overall: the manuscript is a bit long. Maybe the methods section can be cut back in size.

Reply 3: It is really true as the reviewer suggested that the manuscript is a bit long, especially the method part. And we are sorry for not present it in a more concise way. Therefore, we have reduced the length of the method section according to the reviewer's suggestion.

Change in the text: We have cut back the method part to make it more concise. Due to the deleted content is very miscellaneous, we will not elaborate on them one by one here.

Special thanks to you for your good comments.