Peer Review File

Article Information: https://dx.doi.org/10.21037/jtd-24-978

Reviewer A

The authors have constructed a Pathomic model based on various pathological aspects and pathway genes related to TIGIT expression, presenting highly intriguing data on the prognosis of LUAD. While this study is highly original, there are some concerns that need to be addressed:

The impact of differences in expression as determined by IHC (Figure 2B) on prognosis does not seem to differ significantly from the impact of differences in scores based on the Pathomic model (Figure4C). Since IHC is a much simpler test than constructing a Pathomic model, the merit of building the Pathomic model is unclear. This point should be discussed in greater depth in the Discussion section.

Reply 1: Thanks to the reviewer. The expression of TIGIT in the tumor in Figure 2B of this study was based on RNAseq data. Currently, the detection of TIGIT expression levels can only be done through the following methods: detection of peripheral blood cytokines, detection of mRNA or protein levels based on fresh tissue specimens, and detection based on paraffin tissue specimens. All of these methods are subject to subjective influence of operators, antibodies, and high prices. Take IHC as an example. A large number of studies have found that diagnostic immunohistochemistry tests have performance differences between laboratories and cannot quantitatively and objectively evaluate the sensitivity of immunostaining [PMID: 29595317]. For example, in malignant mesothelioma, IHC specificity is not high and there is no exclusive specific antibody [PMID: 17075297]. However, H&E stained sections are necessary for clinical diagnosis and are the most easily accessible image data. Artificial intelligence is gradually being applied to pathology, causing great changes in pathology. Pathomics refers to the conversion of pathological images into high-fidelity, high-throughput mineable data based on artificial intelligence, covering quantitative features such as texture features, morphological features, edge gradient features, biological characteristics, etc., and is used for quantitative pathological diagnosis, molecular expression and disease prognosis. [PMID: 35738057; PMID: 33801859; PMID: 31417906] The pathomics model can digitize tissue slice information. By extracting digital features that cannot be observed by eye, it can analyze the characteristics of diseased tissues to a greater extent and has a good ability to predict the microscopic or molecular phenotype of tumors. For example, Chen S et al. used the pathomics model to predict the prognosis of bladder cancer, and the model had good prediction performance [PMID: 33931925].The AUC values of the training set and the validation set of the pathomics model in this study were both more than 0.7, indicating that the prediction performance of the pathomics model constructed in this study was good. We have modified our text as advised (see Page 13-14, line 306-329).

Changes in the text: Currently, the detection of TIGIT expression levels can only be done through the following methods: detection of peripheral blood cytokines, detection of mRNA or protein levels based on fresh tissue specimens, and detection based on paraffin tissue specimens. All of these methods are subject to subjective influence of operators, antibodies, and high prices. Take IHC as an example. A large number of studies have found that diagnostic immunohistochemistry tests have performance differences between laboratories and cannot quantitatively and objectively evaluate the sensitivity of immunostaining[17]. For example, in malignant mesothelioma, IHC specificity is not high and there is no exclusive specific antibody[18]. However, H&E stained sections are necessary for clinical diagnosis and are the most easily accessible image data. The pathomics model can digitize tissue slice information.

By extracting digital features that cannot be observed by eye, it can analyze the characteristics of diseased tissues to a greater extent and has a good ability to predict the microscopic or molecular phenotype of tumors. For example, Chen S et al. used the pathomics model to predict the prognosis of bladder cancer, and the model had good prediction performance[19]. We constructed an objective batch pathomics prediction model for TIGIT expression in lung adenocarcinoma, with an AUC of 0.735 for the training set and 0.738 for the validation set. Based on the current criterion that AUC>0.7 is a good performance[20], the pathomics prediction model for TIGIT expression in lung adenocarcinoma has good prediction performance. In addition, the calibration curve shows that the model has a good calibration degree; DCA shows that the model has a high clinical net benefit. Through pathomics, objective, batch, and accurate prediction of TIGIT expression can be achieved**.**

EMT-related factors have been reported as poor prognostic factors in many cancers including lung cancer. Considering that TIGIT expression strongly correlates with EMT factors, which are known to be poor prognostic indicators, how do the authors explain the inconsistency with TIGIT being a favorable prognostic factor? This should be addressed in the Discussion.

Reply 2: Thank you for the reviewer's suggestions. We believe that it is necessary to discuss the significance of TIGIT for the prognosis of lung adenocarcinoma. In our study, we found that high expression of TIGIT (HR=0.65, 95%CI 0.442-0.954, P=0.028) was a protective factor for OS. In previous studies, the effect of TIGIT on prognosis was analyzed in 33 types of cancer, and it was found that TIGIT played a completely different role in different cancers. High expression of TIGIT was associated with poor prognosis in Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), low-grade glioma (LGG), and Uveal Melanoma (UVM), and with good prognosis in breast cancer (BRCA), head and neck squamous cell carcinoma (HNSC), and cutaneous melanoma [PMID: 34795387]. A meta-analysis of solid tumors in East Asian populations found that no effect of TIGIT on tumor OS prognosis was found in 8 studies, and TIGIT was found to be a risk factor for tumor OS prognosis in the remaining studies [PMID: 36211383]. Therefore, the role of TIGIT in tumors may be affected by tumor type and regional population, which still needs further research and exploration. Although the role of TIGIT in cancer is still controversial, it is undeniable that TIGIT has become an important indicator for lung cancer prognosis and immunotherapy [PMID: 37030062]. We constructed an objective batch pathomics prediction model for TIGIT expression in lung adenocarcinoma, with an AUC of 0.735 for the training set and 0.738 for the validation set. Based on the current criterion that AUC>0.7 is a good performance [PMID: 32558385, 35277527], the pathomics prediction model for TIGIT expression in lung adenocarcinoma has good prediction performance. In addition, the calibration curve shows that the model has a good calibration degree; DCA shows that the model has a high clinical net benefit. Through pathomics, objective, batch, and accurate prediction of TIGIT expression can be achieved. In the future, it is expected to provide a basis for guiding TIGIT clinical targeted therapy by screening people with potential benefits for TIGIT targeted therapy. We also supplemented this in the Discussion (see Page 14-15, line 360-377).

Changes in the text: Although EMT usually predicts a poor prognosis in the vast majority of studies, how TIGIT regulates EMT needs further mechanistic studies. We believe that it is necessary to discuss the significance of TIGIT for the prognosis of lung adenocarcinoma. In our study, we found that high expression of TIGIT (HR=0.65, 95%CI 0.442-0.954, P=0.028) was a protective factor for OS. In previous studies, the effect of TIGIT on prognosis was analyzed in 33 types of cancer, and it was found that TIGIT played a completely different role in different cancers. High expression of TIGIT was associated with poor prognosis in Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), low-grade glioma (LGG), and Uveal Melanoma (UVM), and with good prognosis in breast cancer (BRCA), head and neck squamous cell carcinoma (HNSC), and cutaneous

melanoma[28]. A meta-analysis of solid tumors in East Asian populations found that no effect of TIGIT on tumor OS prognosis was found in 8 studies, and TIGIT was found to be a risk factor for tumor OS prognosis in the remaining studies[29]. Therefore, the role of TIGIT in tumors may be affected by tumor type and regional population, which still needs further research and exploration. Although the role of TIGIT in cancer is still controversial, it is undeniable that TIGIT has become an important indicator for lung cancer prognosis and immunotherapy.

Reviewer B

The **Background** in the **Abstract** should describe relevant background information. For example, what is known and unknown. While the current one contains only the object. Please modify the abstract.

Reply: We have modified the Background as: Traditional diagnostic methods have limited efficacy in predicting the prognosis of lung adenocarcinoma (LUAD), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) is a new biomarker. This study aimed to evaluate TIGIT expression as a LUAD biomarker and predict patient prognosis using a pathological feature model.

Figures and tables

(1) Please add an explanation for "***" in the legend of Figure 2.

Reply: We have added explanation for "***" in the legend of Figure 2.

(2) It is highly suggested to unify the data in the figure and the main text. 0.63[0.44 to 0.92]
(HR=0.634, 95% CI: 0.439–0.915, P=0.015)

Reply: Thanks for your careful work, we have modified the data.

(3) A table header is needed for the first column of Table 2.

Reply: We have added the head for the first column of Table 2.