Peer review file

Article information: http://dx.doi.org/10.21037/jtd-20-2909

Comment 1: The manuscript should be shortened as there are many repetitions specifically in the discussion that mainly repeats the results; the introduction could also be shortened.

Reply 1: We appreciate the suggestion. We have modified the text as advised.

Changes in the text: We restructure the "Introduction" and the "Discussion" the modifications are in paragraph 2 (lines 425-434) and paragraph 4 (lines 451-452); we removed repetitions in this item.

Comment 2: Some of the facts presented in the intro are pertaining to NSCLC and not MM; please clarify.

Reply 2: We appreciate the note. We have modified the text as advised to clarify this point. Thank you.

Changes in the text: We restructure the "Introduction", this information was added in paragraphs 1 and 2.

Comment 3: For IHC – were the stains tested on whole tissue sections to ensure that PHLDA family members stain evenly and staining is not patchy? If staining is patchy TMAs potentially harbor selection bias.

Reply 3: We agree with the reviewer. Thank you for this comment. The stains were tested on all the tissue previously. Moreover, each TMA was constructed from the primary resected tumors using three regions, obtained from the center, middle, and periphery. Staining homogeneity resulted in a low standard deviation between samples from the same tumor, and even between different samples, for all PHLDAs. In the new version of the manuscript, we added this important statement in the Methods sections (item 3.1).

Changes in the text: Methods, item 3.1, lines 174-177.

Comment 4: Please provide the clones of the antibodies used for IHC.

Reply 4: Thank you for this comment. The solicited information has been added in the Methods (item 3.2).

Changes in the text: Methods, item 3.2, lines 184-190.

Comment 5: Why was $p<=0.05$ used as definition of significance – usually $p<0.05$ is used as statistically significant.

Reply 5: Thank you for the note. We correct the points where p <= 0.05 was wrongly used, all changes are highlighted in the text and table 3.

Changes in the text: Methods, item 1, line 146 and item 4, line 259; Results, item 1.1, lines 275-278; Legend of Supplementary figure 2, line 674; and Table 3.

Comment 6: Please explain what you mean by "LUAD squamoid alveolar lineage type". Similarly, what is meant by "magnoid", "bronchioid" and "squamoid" expression type?

Reply 6: We totally agree with the reviewer: "magnoid", "bronchioid" and "squamoid" expression types of LUAD usually are not terms to classify LUAD by us, pulmonary pathologists. However, using genome-wide gene expression profiling, LUAD has been divided into intrinsic molecular subtypes by many investigators (Wilkerson et al., 2012; Sos et al., 2009; Takeuchi et al., 2006; Beer et al., 2002; Bryant et al., 2010; Motoi et al. 2008; Song et al., 2018), including a meta-analysis (Ettinger et al., 2010), and data mining, in which these investigators named the molecular subtypes: "Bronchioid", "Magnoid", and "Squamoid". The subtypes represent the main naturally occurring patterns of LUAD gene expression and separate tumors following different functional pathways, such as proliferation in "Magnoid" and development in "Bronchioid". As in our work, we used data mining we preserved the nomenclature used by the authors. In the new version of the paper, we included a footnote with this explanation with the references. Thank you for the question.

Wilkerson et al. Differential Pathogenesis of Lung Adenocarcinoma Subtypes Involving Sequence Mutations, Copy Number, Chromosomal Instability, and Methylation. Plos One. 2012; 7(5):e36530.
Sos et al. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. J Clin Invest. 2009, 119(6):1727–1740.
Takeuchi et al. Expression Profile–Defined Classification of Lung Adenocarcinoma Shows Close Relationship With Underlying Major Genetic Changes and Clinicopathologic Behaviors. J Clin Oncol. 2006; 24(11):1679–1688.
Beer et al. Gene expression profiles predict survival of patients with lung adenocarcinoma. Nat Med. 2002; 8:816–824.
Bryant et al. Clinically Relevant Characterization of Lung Adenocarcinoma Subtypes Based on Cellular Pathways: An International Validation Study. Plos One. 2010; 5:e11712.
Motoi et al. Lung Adenocarcinoma: Modification of the 2004 WHO Mixed Subtype to Include the Major Histologic Subtype Suggests Correlations Between Papillary and Micropapillary Adenocarcinoma Subtypes, EGFR Mutations and Gene Expression Analysis. Am J Surg Pathol. 2008; 32(6):810–827
Song et al. High PITX1 expression in lung adenocarcinoma patients is associated with DNA methylation and poor prognosis. Pathol Res Pract. 2018; 214(12):2046-2053.
Ettinger et al. Non-small cell lung cancer. J Natl Compr Canc Netw. 2010; 8:740–801.

Changes in the text: Table 2.

Comment 7: Which histologic subtypes of MM were included and did the histologic subtype correlate with expression of PHLDA? If biphasic was included how was ensured that both components were equally represented in the TMAs? If histologic subtype correlates with PHLDA expression, are the described significant findings independent of the histologic subtype?

Reply 7: Dear reviewer, the point that was highlighted in this question is extremely relevant. There are current debates by MM investigators, such as the one presented by

Salle et al. (2020) on the MM biphasic histotype, that this would be just a more aggressive form than MM epithelioid histotype. Therefore, in our study, we considered only the epithelioid and sarcomatoid histotypes, precisely in order to avoid such confusion. In our results, we could see that the expression of PHLDAs was tumor agnostic or independent of the histological subheading [Please see the reference Looney et al., 2020]. We appreciate the possibility of this discussion and add information about histological subtypes to the text.

Salle et al. Comprehensive Molecular and Pathologic Evaluation of Transitional Mesothelioma Assisted by Deep Learning Approach: A Multi-Institutional Study of the International Mesothelioma Panel from the MESOPATH Reference Center. Journal of Thoracic Oncology. 2020, 15(6):1037-1053.
Looney et al. Tumour-agnostic therapies. Nature Reviews Drug Discovery. 2020, 19:383-384.

Changes in the text: Methods, item 3.1, line 169.

Comment 8: In regards to expression – why was PHLDA expression compared between MM and LUAD, what was the aim for that comparison and what do the results might imply?

Reply 8: Thank you for this important comment. MM and LUAD share the common fact that both are potentially highly aggressive tumors; the difference is the broader scope of therapeutic possibilities nowadays available for LUAD, while MM still does not have a broad arsenal. In fact, besides surgery for early stages, there are not many life sparing (or increasing disease-free interval) possibilities. Considering the broader aspect of target drugs for LUAD and the broad scope of PHLDA-drug interaction network, our expectation was to extrapolate the use of PHLDA immunohistochemistry as a possible tumor agnostic biomarker (Looney et al., 2020), offering increased possibilities to cancer that is not well served on personalized medicine options. The expression of PHLDA2 and PHLDA3 in both tumors may be a hint that these tests may serve as a potential tumor agnostic biomarker.

Looney et al. Tumour-agnostic therapies. Nature Reviews Drug Discovery. 2020, 19:383-384.

Changes in the text: Introduction, paragraph 4 (lines 111-116).

Comment 9: In 2.3 what was included in the multivariate analysis-histologic subtype? Stage?

Reply 9: We appreciate this pertinent question very much, thank you. Currently, there are two important criteria for including variables in multivariate analysis: 1) using the p-value obtained in Cox's univariate analysis, and 2) using classically accepted criteria with impact on the risk of death and overall survival, namely staging and histological types, as co-dependent variables of the statistically significant variables obtained in the individual or univariate analysis. As shown in Table 6, we did not obtain statistical significance in the univariate for all variables. We tested the multivariate to see if the dependent variables would depend on the control of the model (co-dependent) by the classically accepted variables with an impact on the risk of death and overall survival, namely staging and

histological types, and in fact, we have demonstrated that it is. This important statement was included in the Methods (item 4) and better explained in Results section.

Changes in the text: Methods, item 4, lines 246-256.

Comment 10: Figures 1A and C - it is difficult to imagine that there is a significant difference between normal and tumor.

Reply 10: Dear reviewer, thank you for this note. Although the comment is pertinent, the data shown in Figure 1A and C were downloaded exactly as presented in the TCGA database and on the UALCAN platform. The tool itself generates the graphs and calculates the *P*-values automatically. Please, follow the link for verification: <http://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=PHLDA1,PHLDA2,PHLDA3&ctype=LUAD>. We appreciate the possibility to answer about this.

Changes in the text: No changes were made to the text.

Comment 11: The interpretation of the IHC staining of PHLDA expression is difficult based on figure 6. What is the true staining, what is background; there appears a high background and staining does not appear convincing, at least not at the power that is provided in the figure; maybe a higher power picture would be better? Figure 6I is described as strong and diffuse p53 staining – this staining is only seen in a subset of tumor cells, so it is not diffuse; also no real staining observed in MM.

Reply 11: We totally agree with the referee's comment. The magnification of the picture in Figure 6 doesn´t allow differentiation between tumor cell staining and background. Then we took new photos at 400X magnification and included an inset at 1000X magnification for a better interpretation of the IHC staining of PHLDA expression. The inset picture allows better identification of cytoplasmic staining with perinuclear accentuation and dots patterns in tumor cells. According to better visualization of PHLDAs expression patterns, we also modified the Methods section and Results. We hope that now the referee will be able to properly interpret the PHLDA staining pattern. Thank you.

Changes in the text: Methods, item 3.2, lines 193-195; Results, item 2.1, lines 365-373; and the Figure 6 legend, lines 651-664.

Comment 12: Conclusion shouldn't be about NSCLC as only LUAD were studied, not squamous cell carcinomas.

Reply 12: We thank the reviewer for the note. The same has been corrected in all relevant points of the work.

Changes in the text: Discussion, paragraph 8, lines 485, 487 and 490; Abstract, line 81.