Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-24-28

Reviewer A

The authors used the TCGA mesothelioma RNAseq data set to investigate the prognostic values of DMD gene in mesothelioma. The study is novel, identifying that high Dp71 and high total DMD expression was associated with poorer overall survival.

My minor comments are as follows:

Comment 1: The authors should define DMD (e.g. Duchenne muscular dystrophy) within the abstract.

Reply 1: DMD was defined in the abstract

Changes in the text: (line 23).

Comment 2: It is unclear how identifying DMD transcripts will inform treatment strategies as mentioned in the abstract and the highlights box. The authors should include some more information on how identifying DMD transcripts will inform treatments strategies.

Reply 2: We greatly appreciate this suggestion.

Changes in the text: Sentence (lines 50-52) expanded to read: Identifying patients at risk of poor survival based on DMD transcript expression can guide treatment strategies in mesothelioma, informing decisions regarding treatment intensity, follow-up schedules, eligibility for clinical trials, and end-of-life care planning.

Also, in the Highlight Box (bottom: Expression of specific DMD transcript identifies patients at risk of poor survival, and therefore should be explored as a predictive biomarker to inform treatment intensity, follow-up schedules, eligibility for clinical trials, and end-of-life care planning).

Furthermore, we have elaborated these points in the Strength and Limitations chapter (Lines 385-389 were added), and also in Conclusions (lines 425-430).

Comment 3: It would be helpful to include a sentence of two on the cohort e.g. number of cases analysed, number of cases that were epithelioid vs non-epithelioid (biphasic, sarcomatoid and NOS), gender age, at the beginning of the results section or within the methods.

Reply 3: Indeed, we are sorry this was not described clearly.

Changes in the text: This information has now been added to the beginning of the Results section (lines 188-193).

Comment 4: It may be useful to define cut off values for low and high expression within Figure 3.

Reply 4: The cut-off values have been added to Figure 3, and a description has been added to the figure caption.

Changes in the text: The following has been added to Figure 3 caption “The cut-off values used to classify patients into high and low expression groups as determined by the X-tile software are also displayed. The unit for the total *DMD* dataset is $\log_2$(norm_count +1) and the unit for the datasets of individual transcripts is normalised RSEM.”.

Comment 5: Possible typographical error line 315 - “informs affects cancer”?
Reply 5: The text in line 330 reads “the expression of Dp71 isoforms affects cancer”
Changes in the text: None

Reviewer B

This paper aims to analyze DMD as a marker for prognosis in mesothelioma. This is the first study to attempt to see if DMD gene mutations have a role in mesothelioma. While the paper makes a compelling argument to look further into this gene, there are a few items to address:

Introduction
Comment 1: Lines 62-63: No citation for the incidence of mesothelioma
Reply 1: This omission was corrected.
Changes in the text: The citation has been added (line 63).
Comment 2: Line 66: There have been other studies that have made advances in understanding the molecular basis of mesothelioma (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7536355/)
Reply 2: This work has now been included.
Changes in the text: The following was added: “despite that substantial advances have been made in understanding the molecular biology of mesothelioma (2) … (lines 66-67). and references were updated.
Comment 3: Line 82: Extra comma between “include, age”
Reply 3: Error corrected.
Changes in the text: This comma was removed (line 83).
Comment 4: Line 97: Would be helpful to explain what types of cancers have shown DMD gene expression is a predictor of survival
Reply 4: The tumour types have been described.
Changes in the text: The following was added “various tumours has been highlighted, including different carcinomas, haematological malignancies, and low-grade gliomas” (lines 98-99), and references have been updated.
Also, in line 99, we modified the sentence to reflect that even larger gene has recently been identified.

Methods
Comment 5: Line 126: UCSC should be spelled out
Reply 5: This was corrected.
Changes in the text: UCSC was spelled out (line 128).
Comment 6: Methods- Data Acquisition: How were demographics and histology of the patients collected? I am assuming this was from the TCGA database, but this is not explicitly stated in the methods section
Reply 6: This was corrected.
Changes in the text: This has now been added (lines 127-128).
Comment 7: Line 145: Please specify version of GraphPad Prism
Reply 7: This was corrected.
Changes in the text: The version was specified (line 147).

Results

Comment 8: Line 187-189: Please provide values to explain what you mean by strong, moderate, weak correlation

Reply 8: This omission was corrected.

Changes in the text: The values were added in the text (lines 203-208).

Comment 9: Line 199-201: This would be better suited at the start of results so we know how many samples from each histiotype were examined.

Response 9: We agree with this suggestion.

Changes in the text: The sentence has been moved to the beginning of the Results section (lines 188-193). Additional information about the cohort have also been added.

Comment 10: Line 202-204: What were the average survival times

Reply 10: This information has now been provided.

Changes in the text: The median survival times have now been added in the text (lines 213-214 and lines 218-220). In addition, these are also shown in Figure 3A.

Comment 11: Line 219-233: This portion of the results doesn't seem to relate to mesothelioma, and is only briefly mentioned in the conclusion. More information on how these pathways are relevant to mesothelioma should be included.

Reply 11: The relevance of these pathway alterations to mesothelioma has now been addressed explicitly in the Discussion.

Changes in the text: Specific information on how these pathways are relevant to mesothelioma have been provided, and relevant references were added (Lines 335-338 and 345-347).

Comment 12: Line 236-238: Was this information about NK cells and M2 macrophages from the database? It's not clear in the methods.

Reply 12: We apologize for this lack of clarity. This has been addressed.

Changes in the text: (lines 250-259). This method has now been described in details in the Methods section (line 179-182).

Comment 13: Figure 1: I'm not sure if this needs to be included in the paper versus a supplemental

Reply 13: We believe that the complexity of the DMD gene warrants inclusion of a figure in the text to enhance readers' understanding. However, should the Editors concur with the Reviewer's suggestion that it is unnecessary, we are open to relegating it to a supplemental figure.

Changes in the text: Currently None

Conclusion

Comment 14: Lin 307-309: This makes a good point for a limitation of the paper- nothing knowing the expression of Dp71 in benign pleura. This would be good to include in the section regarding Strengths and limitations of the paper.

Reply 14: This was addressed, as suggested.

Changes in the text: This limitation has now been described in the Strength and Limitations section (lines 385-389).

Comment 15: 4.4 Explanations of findings: This portion makes the claim that DMD gene transcription is good sole biomarker for mesothelioma because of its multi-faceted nature. It would be good to explain more details of the Human Molecular Genetics paper that is under

review.

Reply 15: The point we are making is that changes in the *DMD* gene expression in mesothelioma can be a good biomarker because of their multiple isoforms having multiple functions. Firstly, they can reflect changes in the TME (e.g. the varying proportion of different TME cells, which express different isoforms). Secondly, levels of DMD transcripts can reflect secondary and/or compensatory differences in mesothelioma that affect survival. Finally, DMD expression can be directly responsible for changes in mesothelioma biology. This latter possibility we refer to when mentioning the Hum Mol Genet manuscript (attached for reviewers' perusal). We describe there that CRISPR-Cas9 ablation of the specific Dp71 splice-variant altered aggressiveness of a tumour cell line expressing this isoform.

However, appreciating that this is still an unpublished paper, we have additionally cited published studies on the role of dystrophin in tumour biology.

Changes in the text: The revised text reads: "The direct link is further corroborated by functional studies illustrating the dystrophin role in tumours, and that it varies depending on the tumour type (21,22,46,48,49). Additionally, our recent work has extended this this role to specific splice variants (Hum Mol Genet, under review)." (lines 413-415).

Additional changes:

- The effect of tumour stage on patient survival in this cohort have been added to Results (Lines 279 and 280) and Supplementary Figure 4 has been updated to reflect this addition.
- The Limitations have been updated.
- DMD gene has been found to be the second largest human gene. This fact was corrected in the revised manuscript (lanes 99 and 406)