Peer Review File

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Reviewer Comments: The authors here investigate whether the expression of a panel of m6A regulator genes can stratify patient risk. The manuscript is overall good with some concerns outlined below.

Comment 1: The authors multiple times reference "ccRCC", which should presumably be replaced with "TGCT".
Reply 1: Thank you very much for your careful reading and helpful question. There are some mistakes because of our negligence. According to your advice, we replaced "ccRCC" with "TGCT".
Changes in the text: We modified the text as advised.

Comment 2: Line 87: "spermatocytic seminoma" is no longer correct nomenclature; "spermatocytic tumor" preferred instead
Reply 2: Thank you very much for your careful reading and helpful question. According to your advice, we replaced "spermatocytic seminoma" with "spermatocytic tumor".
Changes in the text: We modified the text as advised (see Page 4, Line 84 and 86).

Comment 3: Line 90: "The patients' life expectancy at age 30 was estimated as 45.2 years" unclear which patients are being referred to (all TGCT or a subset) and whether this life expectancy is 45.2 years of age, or 45.2 years following diagnosis.
Reply 3: Thank you very much for your careful reading and helpful question. The life expectancy is 45.2 years of age. We have modified the text to make it clear.
Changes in the text: We modified the text as advised (see Page 4, line 88).

Comment 4: Line 171: How were the final 6 genes selected from the panel of 10?
Reply 4: Thank you very much for your careful reading and helpful question. As we described in Line 169-172, the 10 m6A RNA methylation regulators were selected for further Cox multivariate proportional hazards regression analysis. Finally, 6 genes were identified to construct a risk score. We provided Cox multivariate proportional hazards regression analysis in the Supplement Table1.
Changes in the text: We uploaded the Supplement Table1 (see Supplement Table1).

Comment 5: Line 184: 79 patients in training group + 32 patients in test group = 111 out of 121 patients. Why were these 10 patients excluded from analysis?

Reply 5: Thank you for your careful reading and helpful questions. These 10 patients were excluded from analysis for lack of complete clinical data.
Changes in the text: None

Comment 6: Line 251: Should report a quantitative measure here, eg 5-year PFS, in order to demonstrate magnitude of change
Reply 6: Thank you for your careful reading and helpful questions. 5-year PFS=69% in cluster 1, 5-year PFS=79% in cluster 2. We have added these data in the manuscript.
Changes in the text: We modified the text as advised (see Page 12, line 250-251).

Comment 7: Line 255: Instead of using S stage as a surrogate for serum tumor markers, is there any relationship between the markers individually and cluster assignment?
Reply 7: Thank you for your careful reading and helpful questions. The levels of AFP ($p<0.001$) and hCG ($p<0.05$) increased in cluster1, compared with cluster2. However, there was no significant difference between the level of LDH in cluster1 and that in cluster2. We provided the relationship between the markers (LDH, AFP and hCG) individually and cluster assignment in the Supplement Figure1.
Changes in the text: We uploaded the Supplement Figure1 (see Supplement Figure1).

Comment 8: Line 285 and 298: This should say that the low-risk group had better PFS than the high-risk group
Reply 8: Thank you for your careful reading and helpful questions. There are some mistakes because of our negligence. According to your advice, we modified the text in Line 285.
Changes in the text: We modified the text as advised (see Page 13, line 285).

Comment 9: Line 294: Evaluating this risk score using the entire TCGA dataset is inappropriate since the training data set comprises 2/3 of this test data set. Further, it is redundant, as authors have already demonstrated performance using a test set in the previous section, and use an independent data set later in manuscript.
Reply 9: Thank you for your careful reading and helpful questions. According to your advice, we have removed the validation of the prognostic signature in the entire TCGA cohort. However, we retained the identification of the independent prognostic factors in the entire TCGA cohort.
Changes in the text: We modified the text as advised (see Page 14, line 295).

Comment 10: Line 301: Authors should test for significant differences between these curves rather than only reporting AUC values
Reply 10: Thank you for your careful reading and helpful questions. According to your previous advice (Comment 9), we have removed the validation of the prognostic signature in the entire TCGA cohort which included Line 301.
Changes in the text: We modified the text as advised (see Page 14, line 295).

Comment 11: Figure 1B: Missing legend to indicate which color refers to normal and which to tumor
Reply 11: Thank you for your careful reading and helpful questions. The red represents tumor group and blue represents normal tissue group. According to your advice, we added the annotation in the figure legend.
Changes in the text: We modified the text as advised (see Page 24, line 581).

Comment 12: Figure 2: Unclear what an X in a given box indicates. These also obscure coefficients making them hard to read
Reply 12: Thank you for your careful reading and helpful questions. An X represents $p>0.001$, which means there was no statistically significant correlation between two m6A regulatory genes. We have added the annotation of X in the figure legend to make it clear.
Changes in the text: We modified the text as advised (see Page 25, line 585).

Comment 13: Figure 3A-C: The labels on these charts are illegible due to small size and poor resolution
Reply 13: Thank you for your careful reading and helpful questions. According to your advice, we provided a high-resolution image for Figure 3.
Changes in the text: We provided a high-resolution image as advised (see Figure 3).

Comment 14: Figure 3D: This figure seems to suggest that clusters 1 and 2 are well defined by type (seminoma vs non-seminoma). How does expression of m6A regulatory genes change between histologic type?
Reply 14: Thank you for your careful reading and helpful questions. Actually, Figure 3D suggests that clusters 1 and 2 are well defined by m6A RNA methylation regulators because the TGCT patient cohort were divided into two clusters based on the expression of m6A RNA methylation regulators. We found the expression of most m6A regulatory genes was different between seminoma and non-seminoma tissues (Figure 4).
Changes in the text: None

Comment 15: Figure 3F: Color for stage S2 and S3 appears the same to me. It is unclear what “race = yellow” indicates. Why is age separated at 36 years?
Reply 15: Thank you for your careful reading and helpful questions. According to your advice, we have changed the color for stage S3 in Figure 4 and replaced “yellow” with “Asian” in Figure 4. It is reported that median age of incidence was 36 years [Dieckmann, K.P., et al., Testicular Germ-Cell Tumours: A Descriptive Analysis of Clinical Characteristics at First Presentation. Urol Int, 2018. 100(4): p. 409-419.].
Changes in the text: We modified the Figure as advised (see Figure 4).

Comment 16: Figures 5 and 6, panels A and D: y-axis title should clearly reflect that this is progression-free survival, not overall survival
Reply 16: Thank you for your careful reading and helpful questions. According to your advice, we have modified the y-axis titles in Figures 6 and 7.
Changes in the text: We modified the Figure as advised (see Figure 6 and 7).

Comment 17: Figures 5 and 6, panel B: Is this curve significantly different from null? What is the p-value or 95% CI?
Reply 17: Thank you for your careful reading and helpful questions. Both of the p-value<0.01, and this curve is significantly different from null.
Changes in the text: None

Comment 18: Figure 7, panels A and F: y-axis title should clearly reflect that this is progression-free survival, not overall survival
Reply 18: Thank you for your careful reading and helpful questions. According to your previous advice (Comment 9), we have removed the validation of the prognostic signature in the entire TCGA cohort which included Figure 7.
Changes in the text: We removed the previous Figure 7 as advised.

Comment 19: Figure 7B-D: Are these curves significantly different from null? What is the p-value or 95% CI?
Reply 19: Thank you for your careful reading and helpful questions. According to your previous advice (Comment 9), we have removed the validation of the prognostic signature in the entire TCGA cohort which included Figure 7.
Changes in the text: We removed the previous Figure 7 as advised.

Comment 20: Figure 7D: ROC curve for risk score appears abnormal; steps are
Reply 20: Thank you for your careful reading and helpful questions. According to

your previous advice (Comment 9), we have removed the validation of the prognostic signature in the entire TCGA cohort which included Figure 7.
Changes in the text: We removed the previous Figure 7 as advised.

Comment 21: Lines 656, 665, and 675: Caption references blue dots, but figure uses green dots. Order of assignment is also reversed; are red dots indicative of progressing or non-progressing disease?
Reply 21: Thank you for your careful reading and helpful questions. There are some mistakes because of our negligence. The red dots indicated the progress. According to your advice, we have modified the text in the figure legend.
Changes in the text: We modified the text as advised (see Lines 606 and 615).

Comment 22: Table 1: Recommend clarifying “race = yellow”
Reply 22: Thank you for your careful reading and helpful questions. According to your advice, we have replaced “yellow” with “Asian” in Table 1.
Changes in the text: We modified the table as advised (see Table 1).