Peer Review File

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Reviewer Comments

Comment 1: The main limitation, to my view, is that the score is based on extraction and processing of data available in public databases, only (purely in silico-based work), without an attempt to validate it in tissue cohorts of well-defined patients, with full clinical information. Authors show validation by using more than one database, but still do not perform lab work to confirm their findings. This could be easily done by RTqPCR, immunohistochemistry, or other techniques, and would amplify the reach of the work.

Response 1: Thank you for your prompt attention to our manuscript and helpful suggestions. We collected tumor tissues and adjacent normal tissues from 5 TCGT patients and performed RT-qPCR for preliminary verification. We will further implement immunohistochemistry and verify the function of each gene in cells in subsequent experiments. Thank you again for your valuable comments.

Changes in the text: We added the result of RTqPCR in Figure 13.

Comment 2: Introduction, line 59: there are a lot more testicular tumors than these ones (these three may be the most common, but there are many others). Please correct the sentence.

Response 2: Thank you for your careful review and effective help of our manuscript. Based on your suggestions, we have corrected the expression of this sentence. Thank you again for your valuable comments.

Changes in the text: We modified the text as advised (see Page 2-3, Line 61-62).

Comment 3: Introduction: the manuscript is missing at the end of the Introduction a paragraph with the aims.

Response 3: Thank you for your careful review and effective help of our manuscript. Based on your suggestions, We added a paragraph explaining the purpose of this article at the end of the introduction. Thanks again for your valuable suggestions.

Changes in the text: We added the text as advised (see Page 4, Line 93-97).

Comment 4: Introduction: it is a bit too long, and missing literature devoted to apoptosis regulation in TGCTs themselves (instead of other tumor models).

Response 4: Thank you very much for your sincere help and reminder. According to your guidance, we simplified the introduction and added the literature related to the regulation of apoptosis in TGCTs. Thanks again for your valuable

suggestions.
Changes in the text: We modified the text as advised (see Page 3-4, Line 88-92).

Comment 5: Methods: authors should state more clearly what are these "normal tissues" – are they samples of testicular parenchyma from healthy individuals? Or is it tissue adjacent to neoplasms of the testis?
Response 5: Thank you very much for your kind guidance. Normal tissues are samples of testicular parenchyma from healthy individuals. According to your guidance, we have supplemented the source of normal tissues in the article. Thanks again for your help.
Changes in the figures: We modified the text as advised (see Page 4, Line 102).

Comment 6: Authors should explain better how they divided the TCGA cohort: "96 of 142 patients" and "36 cases" – why was the division done as such?
Response 6: Thank you for your careful review and effective help of our manuscript. We must apologize for our mistakes. The text should be "96 of 132 patients". As we pointed out in the method, the 132 eligible TCGT patients with clinical survival time in the TCGA database were randomly divided into two groups at a ratio of 7:3, which were divided into a training group and test group. Thanks again for your valuable suggestions.
Changes in the figures: We modified the text as advised (see Page 4, Line 116-117; Page 7, Line 189-191).

Comment 7: Authors should better explain the risk score equation, namely the values multiplying by the expression of each gene.
Response 7: Thank you very much for your sincere help and reminder. According to your opinion, we have made amendments and supplements to the article. Thanks again for your help.
Changes in the text: We modified the text as advised (see Page 8, Line 212-215).

Comment 8: Authors present across the results DFS for several years; almost all recurrences from testicular germ cell tumors are early recurrences, occurring in the first two years. Recurrences after 2 years are rare, even more after 5 years. I think it is not very relevant for authors to provide data on DFS at 10 years, for instance.
Response 8: Thank you very much for your sincere help and reminder. Based on your suggestion, We deleted the 10-year DFS in Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7, and re-typed the figures. Thanks again for your valuable suggestions.

Changes in the figures: We modified the figures as advised in Figure 3-7.

Comment 9: The Kaplan Meier curves of the authors and other analyses, for instance, would benefit from showing if significance is felt at 2 years after diagnosis.
Response 9: Thank you very much for your sincere help and reminder. Based on your suggestion, we added 2-year time-dependent ROC curve in train group, test group, the entire TCGA cohort and GEO cohort. Thanks again for your valuable suggestions.
Changes in the figures: We modified the figures as advised in Figure 3-7.

Comment 10: T Line 241: "grades" – what do authors mean by "grade" of TGCTs? There is no grading system in TGCTs, differently from other tumor models.
Response 10: Thank you very much for your sincere help and reminder. We apologized for mistakes in our writing. In fact, figure 8 does not contain the stratified information of grade. Thank you very much for your correction, we have made changes in the text. Thanks again for your valuable suggestions.
Changes in the text: We modified the text as advised (see Page 10, Line 267-270).

Comment 11: Figure 6: how do authors interpret that for some of the single genes they are studying, not significant effect on DFS is seen? (LPCAT1, PPP1CA, CHGA).
Response 11: Thank you very much for your sincere help and reminder. Although there is no significant difference in DFS of LPCAT1, PPP1CA, CHGA genes in figure6, we can see that the trend of the KM curve of these genes is consistent with the trend of these genes in Figure9. The reason why there is no difference may be due to the small number of testicular cancer samples in the GEO database. Thanks again for your valuable suggestions.

Comment 12: Figure 7: how do authors define the variable "type" in panel A?
Response 12: Thank you very much for your sincere help and reminder. The variable "type" in panel A stands for pathological type. Thanks again for your valuable suggestions.
Changes in the text: We modified the text as advised (see Page 10, Line 267-270).

Comment 13: Figure 7: why is the HR for stage <1? Higher stage should correlate with recurrence and poor prognosis.
Response 13: Thank you very much for your sincere help and reminder. This may be due to insufficient sample size, which has caused such a deviation. In addition, due to the high sensitivity of testicular germ cell carcinoma to chemotherapy,

there is a higher cure rate even in the advanced stage. This shows that clinical staging may not be a good standard for dividing prognosis. This requires a larger sample size for further verification. Thanks again for your valuable suggestions.

Comment 14: Figure 10: what do authors mean by "stage" and classifications as "S1", "S2" and "S3" – is there less risk for stage 2, higher for stage 3, and even higher for stage I? If this is the case, it may reflect the different treatments that patients undergo. This is an important variable also to consider – these patients have been treated differently according to stage, so comparisons may not be always fair.
Response 14: Thank you very much for your sincere help and reminder. The "stage" represents TNM clinical staging. Our results show that the second stage has a lower risk, the third stage has a higher risk, and the first stage has a higher risk. This may be caused by an insufficient sample size. Based on this consideration, the stage is no longer taken into consideration in the new nomogram, it may be more rigorous. Thanks again for your valuable suggestions.
Changes in the figures: We modified the text as advised (see Figure 10; Page 11, Line 310-312; Page 11, Line 314-317).

Comment 15: Discussion: authors can expand on this. And better discuss other biomarkers described in literature aiming to stratify the risk of TGCT patients: vascular invasion, % of embryonal carcinoma, MIB1 index, CXCL12, etc. Discussion should be more centered on TGCTs and comparison with other tissue biomarkers. Relevant literature and recent studies should be cited.
Response 15: Thank you very much for your sincere help and reminder. Based on your suggestion, We have increased the content of the discussion section, and added discussions on other biomarkers for risk stratification of TGCT patients, including vascular invasion, the number of embryonic cancers in the tumor, and some serum biomarkers. Thanks again for your valuable suggestions.
Changes in the text: We modified the text as advised (see Page 13-15).

Comment 16: Written English is ok, but still should be improved, as there are some typos and some grammatical mistakes.
Response 16: Thank you very much for your sincere help and reminder. We are pleased to follow your comments and the manuscript has been extensively revised according to your advice. Besides, our manuscript had been edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at CureEdit (www.cureedit.com). Thank you again for your valuable comments. We look forward to hearing from you.