Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-1179

Reviewer A

Comment 1: The number of figures is numerous, 10, which is quite a significant number. As figures 2, 3 and 4 represent survival data, I suggest making Figures 2A,3A, 4A and 2B-K into one Figure 2. And rest of the figures as Supplementary 1(3B-K) and 2 (4B-K). If the journal's policy favours these multiple figures, keeping it as it is OK.

Reply 1: Considering the Reviewer's suggestion, we have integrated Figures 2A, 3A, and 4A together, changing the Kaplan-Meier survival analysis of Figures 3B-K and Figure 4B-K to supplementary images. Then we have adjusted the numbers of subsequent Figures.

Changes in the text: Line 146: "Figure 2B-K" => "Figure 2 D-M"
Line 147: "Figure 3A" => "Figure 2B"
Line 151: "Figure 3B-K" => "Supplementary Figure 2"
Line 154: "Figure 4A" => "Figure 2C"
Line 156: "Figure 4B-K" => "Supplementary Figure 3"
Line 160: "Figure 5A" => "Figure 3A"
Line 164: "Figure 5B" => "Figure 3B"
Line 172: "Figure 6A" => "Figure 4A"
Line 176: "Figure 6B" => "Figure 4B"
Line 180: "Figure 7A-E" => "Figure 5A-E"
Line 188: "Figure 8A" => "Figure 6A"
Line 189: "Figure 8B" => "Figure 6B"
Line 190: "Figure 8C-E" => "Figure 6C-E"
Line 194: "Figure 9A" => "Figure 7A"
Line 200: "Figure 9B" => "Figure 7B"
Line 207: "Figure 10A" => "Figure 8A"
Line 189: "Figure 10B" => "Figure 8B"

Comment 2: As Nf2 is a tumour suppressor, its high expression in many tumours is counter-intuitive as tumour drivers. The authors should clearly explain the context and meaning of increased expression in that tumour. Whether in some tumours, the correlation of high expression doesn't mean any pathogenic driver role? How does the other co-occurring driver influence this correlation in a tumour type?

Reply 2: Thank you for your comments, which made us realize the shortcomings of our article. NF2, as a tumor suppressor factor, is highly expressed in some patients of various cancers. However, our results found that not all patients have good treatment efficacy and better prognosis with overexpression of NF2. This may be due to the complexity of tumor occurrence and development, as well as the imbalance and crosstalk of multiple signaling pathways in tumors. The high expression of NF2 alone may be difficult to regulate the outcome of tumor occurrence and development. Therefore, our research mainly focuses on the relationship between NF2 and the tumor

immune microenvironment, as well as tumor occurrence and prognosis, in the hope that NF2 can be used as a biomarker of efficacy and prognosis fot patients. Additionally, since our research mainly focuses on bioinformatics analysis methods, we can only analyze whether there is a correlation between the expression levels of genes or proteins, but cannot judge whether there is a causal relationship between them. This is also a deficiency in our research. We will try to conduct the functional basic experiments of NF2 in the future. In addition, we will modify the content of the discussion to elucidate the relevant mechanism of NF2 as a tumor suppressor, and further elaborate on the bioinformatics methods and the shortcomings of our research.

Changes in the text: Line 220: "Previous studies have shown that *NF2* can mediate intracellular signaling effectors, membrane proteins, interactions between actin filaments, and contact-dependent growth inhibition in different stages of organism development" => "The activation of developmental signaling pathways is a similarity between embryonic tissue growth and tumorigenesis. WNT/ β, TGF- β、 RTK, Hippo, and Notch pathways are key participants in normal developmental biology. Loss of restriction on developmental related signaling pathways can cause damage to tissue development, manifested as developmental syndrome. Similarly, by promoting cell proliferation, migration, and stem cell like phenotype, the activity imbalance of these pathways can promote cancer occurrence and progression. As a spatiotemporal-dependent manner, NF2 contributes to either activation or inhibition of developmental pathways in order to maintain cell integrity, tissue organization, and adequate different stages of organism development."
Line 311: added "In addition, we found that even as a tumor suppressor, some patients still have poor prognosis when NF2 is highly expressed. This may be due to the heterogeneity and complexity of tumor occurrence and development, as well as the imbalance and crosstalk between various signaling pathways. However, since our research mainly focuses on bioinformatics analysis methods, we can only analyze whether there is a correlation between the expression levels of genes or proteins, but cannot judge whether there is a causal relationship between them."

Reviewer B

Comment 1: What is the relationship between NF2 and tumor-infiltrating immune cells? What role does NF2 play in prognosis in tumor? It is recommended to add relevant content.

Reply 1: We feel great thanks for your professional review work on our article. We agree with your point of view that fully explaining the relevant results during the discussion will help readers better understand our research results. Therefore, we have added discussion content on the relationship between NF2 and prognosis, as well as the relationship between NF2 and immune cells in the discussion section.

Changes in the text: Line 239: added "To investigate the association between NF2 expression levels and prognosis, survival association analysis was performed using Kaplan– Meier survival curves for each type of cancer, including OS, DSS, and PFI. Combining these results, we found that high NF2 expression had a good prognosis in ESCA, LGG, PAAD, GBM, KIRC,

MESO, KIRP, THYM, STAD, OV, and a poor prognosis in ACC, CHOL, LIHC, SARC, SKCM, and TCGT."
Line 259: added "we found that high expression of NF2 is associated with high expression of TH2 cells and the inhibition of pDC cells. In addition, in COAD, OV, and PAAD, high expression of NF2 is correlated with high expression of various immune cells."

Comment 2: Some fonts need to be enlarged, as shown in Figures 5,7,8,9.

Reply 2: Thank you for your comments, we agree with your point of view that maintaining clear fonts in the figures is beneficial for readers to better understand the research. However, due to the characteristics of pan-cancer analysis, there is too much content in every figure. While ensuring reasonable layout and figure size, we had to only slightly increase the size of the fonts and made some of the fonts bold. If the fonts size still cannot meet the publishing requirements, we will seek for editors' advice to find a way to make the figures meet the publishing requirements

Changes in the text: Increase the size of fonts in Figure 5,7,8,9 (Now is Figure 3,5,6,7)

Comment 3: In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 3: It is really true as your comments that it is necessary to clearly indicate t limitations of prior study and the clinical significance of our study. We agree that clear and effective expression can help readers better understand our research content. Based on your suggestion, we have added some appropriate content in the last paragraph of the introduction.

Changes in the text: Line 54-59: "Nevertheless, the role of *NF2* in cancer has not received sufficient attention". => "The occurrence of *NF2* mutations can affect tumor progression or prognosis. However, there is still a lack of larger researches to evaluate the inactivation of *NF2* in cancer. We still need to better understand the role of *NF2* in tumor development and progression. The fact that the absence of *NF2* mainly leads to the formation of tumors in Schwann, meningeal, and ependymal cells, while other cell types, although commonly expressing merlin in normal tissues, do not undergo transformation, indicating that tissue specific molecular background and tumor microenvironment dependence need further clarification".
Line 67-69: added "The combination of molecular therapies around NF2 may become a successful treatment method. Through further research and better understanding of NF2 related molecular cross signaling, may determine the optimal treatment strategy and achieve personalized precision medicine".

Comment 4: It is recommended to increase the functional experimental study of the NF2 gene.

Reply 4: We agree with your comments that functional experiments are also necessary. Your suggestion provides a direction for our next research. Unfortunately, due to the limited time and funding, we did not supplement experimental validation. In this study, we aimed to explore the molecular mechanism of NF2 expression and its relationship

with tumor immune microenvironment in pan-cancer. Thus, we mainly focus on the results of bioinformatics analysis. We have added this deficiency to the limitation section, and we will try to validate the accuracy of this pan-cancer analysis with external experiments in indicated cancer types.

Changes in the text: Line 319: added "and conduct functional experiments on NF2".

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "A phase II study of everolimus in patients with advanced solid malignancies with TSC1, TSC2, NF1, NF2 or STK11 mutations, J Thorac Dis, PMID: 34422335". It is recommended to quote the article.

Reply 5: Thank you very much for your comments. We have read the article you provided and found that it is highly relevant to our research. We have extracted the main content of the article and added it to the introduction section.

Changes in the text: Line 52-53: added "In addition, some clinical trials have been conducted to explore the prognostic impact of NF2 mutations on solid tumor patients(12)."

Comment 6: It is suggested to increase the in-depth study on the function of NF2 in the occurrence and metastasis of different cancers, which may make this study more complete.

Reply 6: We agree that more studies would be useful to understand the details, after carefully evaluating the funding and experimental conditions required to complete these supplementary works, we found it beyond the scope so that we could not carry out the work. However, we believe the present results can still support the conclusion of this paper. Therefore, we have added this deficiency to the limitation section, and we suggest that supplementary experiments be included in future papers.

Changes in the text: Line 317: "validate the accuracy of this pan-cancer analysis with external experiments in indicate cancer types" => "increase the in-depth study on the function of NF2 in the occurrence and metastasis of different cancers"