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Reviewer A

The manuscript, titled "CTNND2 gene expression in melanoma tissues and its effects on the malignant biological functions of melanoma cells," investigates the expression patterns and biological role of CTNND2. Through analysis of patient samples, cell lines, and in vivo experiments with CTNND2-knockdown A375 cells, the authors demonstrate that CTNND2 is upregulated in melanoma tissues, plays a role in cell adhesion via the FAK and MEK1/2/ERK1/2 signaling pathways, and influences tumor growth in xenografted cells.

This study effectively highlights CTNND2's role in melanoma progression through adhesion mechanisms, supporting their hypothesis on FAK and MEK1/2/ERK1/2 pathway involvement. Further research may be needed to investigate CTNND2's specific role in tumor growth, particularly by examining its interactions with p53 and other oncogenes. Minor revisions are suggested for gene and protein name formatting. Overall, this well-structured manuscript provides promising findings and is suitable for publication in this journal.

Reply: Thank you for your recognition of our manuscript and for the high evaluation of our research content. We also appreciate your valuable suggestions for revisions. We have adjusted the formatting of gene and protein names in the manuscript to ensure they meet the required standards. Additionally, regarding your suggestion to further explore the specific role of CTNND2 in tumor growth, particularly its interactions with p53 and other oncogenes, we consider this a very valuable research direction. In our future studies, we plan to investigate the mechanisms of CTNND2 in greater depth, especially in terms of its role in regulating tumor growth and its interactions with other key signaling molecules, to further advance knowledge in this field.

Change in the text: The entire text has been reviewed to ensure that gene names are italicized, while protein names are not italicized.

Reviewer B

The paper titled "CTNND2 gene expression in melanoma tissues and its effects on the malignant biological functions of melanoma cells" is interesting. The expression of the CTNND2 gene is increased in melanoma tissues, which enhances the ability of melanoma cells to proliferate both in vivo and in vitro. Additionally, the CTNND2 gene is crucial in controlling the adhesion process of melanoma cells. This mechanism is associated with the regulation of the FAK and MEK1/2/ERK1/2 signaling pathways. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There are a variety of genes or drugs that can regulates melanoma progression. Why did the author choose CTNND2 for research? Please describe the reason.

Reply: Thanks for your suggestions. Previously, we performed RNA-sequencing to predict the differential expression of mRNAs in clinical melanoma and paired paracancerous tissues (n=5). Following screening criteria (fold change ≥ 1.5, p-value < 0.05, false discovery rate < 0.05), the results showed that 865 genes were up-regulated while 631 genes were down-regulated. We identified that CTNND2 was significantly upregulated in melanoma tissues.

Change in the text: Page 10/line 316-320.

2) Cancer cells often reprogram their metabolism to effectively support cell proliferation and survival. Please analyze whether and how these metabolic changes promote the proliferation and migration of tumor cells based on existing knowledge and literatures. What is the most important role played by CTNND2 in this process?

Reply: Your suggestions are not only very helpful for this paper, but also beneficial for our future work in this field. Previous studies have showed that CTNND2 reveals inducible mutagenesis promoting cancer cell survival adaptation and metabolic reprogramming[1]. In addition, hypoxia-inducible transcriptional factor (HIF1α) can directly upregulate the expression of the gene CTNND2 (codes the protein δ-Catenin), which can stabilize β-Catenin by disrupting the destruction complex, which leads to the activation of Wnt signaling[2]. Base on these studies, we think that CTNND2 may function as a oncogene and paly a critical role in cancer metabolism and progression. So in the following study, We will further these affects.

Change in the text: None.

3) There have been many studies on melanoma. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply: Thanks for your suggestions. Compared with previous studies, our study firstly showed that CTNND2 is significantly upregulated in melanoma tissues compared to adjacent non-tumor tissues, and modulates melanoma cell behavior through the focal adhesion kinase and mitogen-activated extracellular signal-regulated kinase 1/2/extracellular signal-regulated protein kinase 1/2 signaling pathways, but not the phosphatidylinositol 3-kinase/protein kinase B pathway.

Change in the text: page 3/line 94-98.

4) How to grade melanoma based on the content of this study? How to customize different treatment methods for patients with different risks? Suggest adding relevant content.

Reply: Thanks for your suggestions. We have added the following content to the conclusion section: "Clinically, patients who are CTNND2-positive with advanced tumor stage, lymph node metastasis, and tumor ulceration can be considered a high-risk group. For high-risk patients, personalized treatment strategies targeting CTNND2 may be considered, in combination with existing therapeutic

methods to improve treatment efficacy. Future studies will further explore the specific application of CTNND2 as a target for risk stratification and personalized therapy through larger clinical sample sizes."

Change in the text: page 17/ line 554-560.

5) The study of biological processes in this research is too simplistic. It is recommended to supplement the analysis of biological information.

Reply: Thanks for your suggestions. We have added the results of biological information.

Change in the text: Page 10/line 316-320.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Identification of immune-related biomarkers associated with tumorigenesis and prognosis in cutaneous melanoma patients, Cancer Cell Int, PMID: 32508531”. It is recommended to quote this article.

Reply: Thanks for your suggestions. We have cited this article.

Change in the text: reference 6.

7) It is recommended to increase the study of lncRNA or miRNA to regulate the CTNND2, which may make the whole study more complete.

Reply: Thanks for your comments. Previous studies have showed that miRNA can regulated CTNND2 expression. lncRNA EIF3J-AS1 accelerates hepatocellular carcinoma progression via targeting miR-122-5p/CTNND2 axis[3]. But, Hypoxia also induced CTNND2 expression to enhance mice hepatocellular carcinoma progression via Wnt signaling[2]. So we think, the molecular regulatory mechanism of CTNND2 may be different in different types of cancers. In future studies, we plan to further investigate the potential roles of lncRNAs and miRNAs in regulating CTNND2 in melanoma, which could provide deeper insights and enhance the comprehensiveness of this research.

Change in the text: None.

Reference.

[1] J. Nopparat, J. Zhang, J.P. Lu, Y.H. Chen, D. Zheng, P.D. Neufer, J.M. Fan, H. Hong, C. Boykin, Q. Lu, δ-Catenin, a Wnt/β-catenin modulator, reveals inducible mutagenesis promoting cancer cell survival adaptation and metabolic reprogramming, Oncogene 34(12) (2015) 1542-52.

[2] F. Huang, J. Chen, R. Lan, Z. Wang, R. Chen, J. Lin, L. Fu, Hypoxia induced δ-Catenin to enhance mice hepatocellular carcinoma progression via Wnt signaling, Exp Cell Res 374(1) (2019) 94-103.

[3] X. Yang, B. Yao, Y. Niu, T. Chen, H. Mo, L. Wang, C. Guo, D. Yao, Hypoxia-induced lncRNA EIF3J-AS1 accelerates hepatocellular carcinoma progression via targeting miR-122-5p/CTNND2 axis, Biochem Biophys Res Commun 518(2) (2019) 239-245.

Reviewer C

This manuscript investigates the expression of Catenin delta 2 (CTNND2) in melanoma cell lines and tissue samples, and its correlation with patient clinicopathological features. The study demonstrates that suppressing CTNND2 in a melanoma cell line significantly reduces cell proliferation, migration, invasion, and adhesion to extracellular matrix components through the FAK and MEK1/2-ERK1/2 signaling pathways. Additionally, CTNND2 suppression substantially inhibits melanoma growth in a nude mouse model.

1. A significant limitation of this study, as acknowledged by the authors, is its reliance on a single cell line (A375). To further strengthen the hypothesis and conclusions, studying multiple cells lines, and using a complementary approach, such as overexpressing CTNND2 in a cell line with inherently low expression level (e.g., M14), would provide valuable additional support.

Reply: Thank you for your valuable suggestion. Your suggestions are not only very helpful for this paper, but also beneficial for our future work in this field. However, due to limitations in experimental resources and time constraints, we were unable to perform these additional experiments. We greatly appreciate your professional suggestions and will prioritize addressing these aspects in our future studies to further enhance the comprehensiveness of our findings. We have stated the limitations of this study in the conclusion section.

Changes in the text: page 18/line 564-566.

2. Line 295 and 296, “The immunohistochemical results showed that the CTNND2 levels were markedly increased in the melanoma tissue compared to the normal nevus tissue”. In lines 297 and 298 ” The CTNND2 score for the melanoma tissues was higher than that for the pericancerous tissue”.

How many nevus cases have been tested for their CTNND2 expression? Given that melanocytic nevi are the benign counterpart of melanoma, comparing CTNND2 expression in benign nevi to melanoma would provide a more robust control.

Reply: Thanks for your suggestions. The expression of CTNND2 was examined in 10 cases of normal nevus tissues.

Changes in the text: page 10/line 322-323.

What types of cells/structures in normal skin (pericancerous tissue) show CTNND2 expression? Please clarify.

Reply: Thank you for your comment. The CTNND2 is primarily localized in the cytoplasm of keratinocytes and melanocytes.

Changes in the text: page 10/line 320-322.

3. In Table 2, 12 melanoma cases show negative CTNND2 expression. How many different subtypes of melanomas are represented among these 12 cases?

Reply: Thank you for your comment. Among the 12 cases, there were 6 cases of acral lentiginous melanoma, 4 cases of superficial spreading melanoma, and 2 cases of nodular melanoma.

Changes in the text: None.

4. Since clinicopathological and follow up metastatic information is available for these 46 melanoma cases, is it possible to perform a Kaplan Meier survival analysis based on CTNND2 expression for these 46 patients?

Reply: Thank you for your suggestion. Survival curves for overall survival have been established for the two groups.

Changes in the text: page 11/line 346-353; Figure 2.

5. Lines 418 and 419, "transfection with the interfering sequence targeting CTNN2(shRNA-CTNND2-1) significantly reduced the ERK1/2, pERK1/2 and p-MEK1/2 levels in melanoma cells". ERK1/2 levels showed no significant change in figure 6B, therefore please delete "ERK1/2" here.

Reply: Thank you for your comment. "ERK1/2" has been deleted in the revised manuscript.

Changes in the text: Page 14/line 452.