Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-1537

Reviewer A

Comment 1: The known data of B7-H3 should be described more in the introduction, especially the role in tumor metabolism (as indicated in Line 54). Also, the related information of B7-H3 and ENO1 should be added to explain why the authors are interested in this pathway.

Reply: Abnormal aerobic glycolysis and anabolic pathways are a major hallmark of cancer that can provide fuel for tumorigenic processes. Relevant studies have shown that the involvement of B7-H3 in the metabolic disorders of cancer cells, but the mechanism is worth exploring. In the early stage, our research group combined protein Pull-down technique with mass spectrometry (LC-MSMS) technique to explore the proteins that have direct interaction with B7-H3 in lung cancer cell lines. ENO1, as a target protein was selected according to the results of mass spectrometry, and the direct relationship between B7-H3 and ENO1 was confirmed by in vivo and in vitro experiments. At present, the exact relationship between B7-H3 and ENO1, the molecular mechanism of their interaction and the significance of their role in the tumorigenesis and development still need to be further explored. The relevant information has been added to the introduction of the manuscript.

Changes in the text: See Page 4, Line 55-63;Page 5, Line 73-74.

Comment 2: Line 64, should add ref. of previous data.

Reply: References corresponding to data from previous studies have been added

Changes in the text: See Page 5, Line 73.

Comment 3: Please correct unit “h” or “hr” , by checking through the article and using the same pattern.

Reply: We have carefully reviewed the full text and corrected it into a uniform form representing hours(h).

Changes in the text: See Page 9, Line 162;Line167.

Comment 4: Results

- Figure 1A, the authors indicated that the level of B7-H3 was highly expressed in lung cancer. (Line 170) How about other cancers? The data should be explained in more detail.

Reply: As indicated in Figure 1A, the B7-H3 was highly expressed in lung cancer basing on the TIMER 2.0 database. Correspondingly, there was a rising trend in different types of tumors, including Glioblastoma multiforme (GBM), Kidney renal clear cell carcinoma (KIRC) and Stomach adenocarcinoma (STAD),etc. The relevant information has been added.

Changes in the text: See Page 10, Line 188-191.

- Why was the experiment of apoptosis and cell cycle performed in this work? What is the reason? The results demonstrated that B7-H3 promoted cell proliferation but why was not shown in cell cycle results?

Reply: B7-H3 may be involved in tumor progression by regulating the cycle and apoptosis of tumor cells. Therefore, we conducted relevant experiments to clarify the effects of B7-H3 on lung cancer cell cycle and apoptosis. In this study, overexpressed B7-H3 has obvious inhibitory effect on the proliferation of lung cancer cells, but it does not necessarily affect the cell cycle. The specific reasons may involve many factors, such as the activation of cell proliferation signaling pathway, the change of apoptosis regulation mechanism, and the expression of cell cycle regulation proteins. The interaction of these factors may result in increased cell proliferation while apoptosis and the cell cycle remain unchanged. However, the specific situation may vary depending on cell type, environmental conditions, and disease state, among others.

Changes in the text: See Page 14, Line 278-281.

- Line 190 mentioned phenotype changes, please describe more.

Reply: The phenotypic changes we mentioned include the proliferation and migration of lung cancer cells, which have been added.

Changes in the text: See Page 11, Line 211-212.

- Figure 2D, wound healing results are not quite clear, if possible adjust to have better resolution.

Reply: The wound healing experiment was re-performed and the results were added to Figure 2D. Wound healing assays showed that B7-H3 overexpression enhanced the migration ability of SBC5 cells.

Changes in the text: See Page 22, Line 431: Figure 2D.

- Figure 3, please add "A", "B" in the figure

Reply: "A" and "B" has been added in Figure 3.

Changes in the text: See Page 22, Line 432.

- Figure 4 C, how different of all protein bands?

Reply: We are very sorry that we forgot to add the protein bands annotation, and the missing original bands and annotations have been added in the revised manuscript.

Changes in the text: See Page 23, Line 433: Figure 4A-4B.

- Figure 4F, how different of all tumor nodules?

Reply: At the end of the experiment, the mice were sacrificed by neck dislocation, and the tumor nodules were taken. The volume of tumor nodules were calculated according to the formula: $a \times b^2 \times 0.5$ (a, largest diameter; b, perpendicular diameter) every four days to monitor tumor growth. The picture shown is the size of the tumor nodules at the end of the experimental observation.

Changes in the text: See Page 9, Line 174-176.

- Figure 5E, please make a better resolution.
Reply: The wound healing experiment was re-performed and the results were added to Figure 5E.
Changes in the text: See Page 24, Line 434.

5. Discussion
Why overexpression of B7-H3 enhanced ENO1 activity? Please explain the possible mechanism or any pathway involved.
Reply: Through the literature review, we found that B7-H3 and ENO1 had many similarities in tumors. For example, both of them were reported to be overexpressed in many kinds of cancers and promote tumor proliferation and invasion; moreover, they could share the common signal pathway, PI3K/Akt pathway, to influence tumor glycolysis. Zuo et al. used B7-H3 antibody to block the expression of B7-H3 in HeLa cells, detected the expression of c-Myc and LDHA genes related to glycolysis, and found that their levels were significantly reduced, suggesting that c-Myc and LDHA may be the direct downstream pathway of B7-H3. Therefore, B7-H3 may mediate Warburg effect to promote tumor progression by regulating ENO1 activity changes induced by c-Myc and LDHA expression. The relevant information has been added in the discussion.
Changes in the text: See Page 15, Line 299-303.

Reviewer B

The manuscript entitled, "B7-H3 promotes proliferation and migration of lung cancer cells by modulating PI3K/AKT pathway via ENO1 activity" is interesting. The strengths of the manuscript include data demonstrating: 1) Increased expression of B7-H3 is correlated with poor prognosis and decreased overall survival in lung cancer patients; 2) B7-H3 overexpression induces the proliferation and migration of SBC5 cells without affecting the apoptosis and cell cycle distribution; 3) B7-H3 interacts and regulates ENO1 activity and their silencing attenuates cell proliferation and migration via downregulating the activation/phosphorylation of PI3K-p85a and AKT pathway; 4) Blocking ENO1 activity attenuates the phosphorylation of PI3K-p85a and AKT as well as inhibits the cell proliferation and migration. Overall, the studies are nicely designed and executed, and there are a few minor comments

Minor comments:
1. In Figure 4C, please label the immunoblots even though the overall expression/qualification of B7-H3 and ENO1 is given in Figure 4A-B.
Reply: We are very sorry that we forgot to add the protein bands annotation, and the missing original bands and annotations have been added in the revised manuscript.

Changes in the text: See Page 23, Line 433: Figure 4A-4B.

2. Please indicate the number of replicates (for example, the data are mean+/- SD of 3 independent experiments) in each figure legend.
Reply: The number of experiments repeated has been added in each figure legend.
Changes in the text: See Page 25, Line 451-452;460;See Page 26, Line 472;488.

3. The database used for the analysis of B7-H3 in lung cancer should also be included in the method section.
Reply: A public database for analysis of B7-H3 expression in lung cancer has been added to the methods section.
Changes in the text: See Page 5, Line 81-88.

4. Please carefully check the manuscript for grammatical errors.
Reply: We have carefully checked and corrected the grammatical errors in the manuscript.