Peer Review File

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Reviewer A

Comment 1: For the section under 'Conditioned medium and endothelial formation assay,' the authors should provide a snapshot of the tubules that were captured under the microscope.
Reply1: Thank you for your remind, the snapshots of every tubules are now added into supplement files ‘Snapshot of Tubules.doc’.

Comment 2: The first sentence in the Conclusion section is not complete. Check for completion.
Reply2: Thank you for you remind, we have added the content (see line509-510).

Comment 3: Check line 354 in Discussion. It should be 'in' not 'on'.
Reply3: Thank you for your remind, we have modified our text as advised (see line444).

Comment 4: The authors should provide a glossary of abbreviations used in the article or state the full meaning of abbreviations at first mention before using the abbreviations subsequently.
Reply4: Thank you for your remind, we have written all abbreviations in to the file ‘Abbreviations.doc’.

Comment 5: I was thinking if B7-H3 promoted CRC progression through HB-EGF, then it would have been part of the study to show the mechanism or pathway through which this effect comes about. If it is outside the scope of the study, it should be stated.
Reply 5: Thank you for your advice. Regarding the specific pathways and mechanisms of regulation, we have only made a preliminary exploration in the last section, which involves changes in protein levels in the PI3K-AKT pathway. Strictly speaking, inhibition experiments should be conducted to verify each level of this pathway. However, since the differences in protein levels observed this time showed satisfactory effects, we have decided to temporarily include this work in subsequent related project plans.

Comment6: As a significant finding about CRC progression involving B7-H3 and associated proteins have been made, can a statement or recommendation be provided on potential for CRC treatment? What is the overall impact of this novel research?
Reply 6: The specific receptor for B7-H3 is still unclear, but anti-tumor therapies targeting B7-H3 have already developed rapidly. Our research further expands on the non-immune pro-tumor mechanisms of B7-H3. Additionally, combining HBEGF inhibitors with B7-H3 treatment may produce a significant synergistic anti-tumor effect, which is the focus of our further research

Comment 7: How were the authors able to eliminate confounding factors in determining that B7-H3 was the protein molecule involved in upregulating HIF and HB-EGF? Could other molecules be involved in the pathogenesis of the disease?
Reply 7: During the process of tumor progression, the regulatory mechanisms involving HIF1A and HBEGF are complex and intertwined. We primarily identified the key role of B7-H3 in these mechanisms by knocking down B7-H3 expression and using gene chip analysis, and eliminate the other minor interfering factors. Our research constructed colorectal cancer cell lines with stable low expression of B7-H3, and gene chip analysis was conducted on these samples. The analysis revealed that HBEGF is the primary molecule whose expression level decreases when B7-H3 is reduced, and this result was validated through cellular experiments.

Reviewer B

The paper titled “B7-H3 enhances colorectal cancer progression by regulating HB-EGF via HIF-1α” is interesting. B7-H3 may transmit intracellular signals through PI3K-AKT-mTOR-HIF-1α signaling, upregulating HB-EGF. As the final transcription factor of the pathway, HIF-1α regulates the transcription of the HB-EGF gene, thereby promoting HB-EGF expression, which eventually mediates cell proliferation, invasion, and angiogenesis and promotes the progression of CRC. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: What are the aspects of targeted treatment for B7-H3? Suggest adding discussion and analysis of relevant content.

Reply 1: Thank you for your remind, we have added the content as advised (see line 107-115). Changes in the texts: The high expression of B7-H3 in tumors makes it a promising therapeutic target. However, due to the unclear receptors for B7-H3, current therapies mainly focus on for cancer antibody-based therapy. B7-H3 antibody-ADCs (antibody-drug conjugates) and B7-H3 targeted CAR (chimeric antigen receptor) T/NK cells (4,5), which deliver drugs or T/NK cells to precisely kill B7-H3-positive tumor cells, are the currently the safest and most effective treatment strategies, but none have yet entered clinical or large-scale application. Further research on B7-H3's pro-tumor mechanisms, localization, function, and molecular pathways in cancer cells, will potentially expand the possibilities for targeted therapies aimed at B7-H3.

Comment 2: What role does B7-H3 play in metabolic reprogramming, invasion and metastasis, and chemotherapy resistance of colorectal cancer? It is recommended to add relevant content.

Reply 2: Thank you for your remind, we have added the content as advised (see line 395-401. Changes in the texts: Furthermore, B7-H3 plays a significant hindering role in cancer treatment. In colorectal cancer, it promotes high expression of BRC33 in tumor cells, thereby counteracting the DNA damage effects of 5-FU (38). In pancreatic cancer, B7-H3 promotes the production of anti-apoptotic protein Survivin, antagonizing the effects of gemcitabine therapy (39). In other malignancies, B7-H3 employs various mechanisms to resist multiple chemotherapy drugs (40-42), greatly diminishing the effectiveness of chemotherapy and becoming a crucial factor leading to poor prognosis.

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

Reply 3: Thank you for your remind, we have added the content as advised (see line 135-139). Changes in the texts: Our research utilized RNA chips to preliminarily screen and analyze changes in the expression levels and biological functions of various molecules after reducing B7-H3 levels in CRC. Then, we analyzed clinical data to investigate the

relationships between B7-H3, HBEGF, and clinicopathological features. Finally, we explored the role and mechanisms of B7-H3 in promoting tumor growth through the regulation of HBEGF.

Comment 4: What is the correlation between the expression of B7-H3 and the prognosis of CRC patients? How to gain in-depth understanding through bioinformatics? It is recommended to add relevant content.

Reply 4: Thank you for your remind, we have added the content as advised(see line 420-438).

Changes in the texts: In colorectal cancer, B7-H3 serves as a significant biomarker for survival (10). As an immune checkpoint protein, B7-H3 can inhibit the function of anti-tumor immune cells in many ways (31). And in the tumor tissues with high expression of B7-H3, there is a significant decrease in immune cells infiltration and the number of CD8+ T cells in the tumor microenvironment (32,33), further exacerbating poor prognosis. In fact, B7-H3 also shows a variety of abilities that promote tumor progression through non-immunological mechanisms. B7-H3 can enhance tumor cell glucose metabolism through the promotion of the Warburg effect, thereby increasing their proliferative activity (34). Furthermore, B7-H3 enhances tumor invasiveness by promoting the MMP9, VGEFA, JAK/STAT3, or inducing epithelial-mesenchymal transition (EMT) (35-37). This leads to higher rates of lymph node metastasis and later tumor staging in B7-H3-positive patients, consistent with findings from our third section of research. Furthermore, B7-H3 plays a significant hindering role in cancer treatment. In colorectal cancer, it promotes high expression of BRC33 in tumor cells, thereby counteracting the DNA damage effects of 5-FU (38). In pancreatic cancer, B7-H3 promotes the production of anti-apoptotic protein Survivin, antagonizing the effects of gemcitabine therapy (39). In other malignancies, B7-H3 employs various mechanisms to resist multiple chemotherapy drugs (40-42), greatly diminishing the effectiveness of chemotherapy and becoming a crucial factor leading to poor prognosis.

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “A three-phase trans-ethnic study reveals B7-H3 expression is a significant and independent biomarker associated with colon cancer overall survival, PMID: 35070416”. It is recommended to quote the articles.

Reply 5: Thank you for your remind, we have added the content as advised and cited some more relevant articles (see line 117-119, 421, 435). Changes in the texts: In colorectal cancer, B7-H3 serves as a significant biomarker for survival (10). In CRC, B7-H3 promotes the tumor progression (7,8) and associated with recurrence and overall survival (9,10).

Comment 6: What are the relevant characteristics of the tumor microenvironment of CRC? What is the correlation between B7-H3 and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply 6: Thank you for your remind, we have added the content as advised (see line 417-422, 133-135, 508-509). Changes in the texts: In colorectal cancer, B7-H3 serves as a significant biomarker for survival (10). As an immune checkpoint protein, B7-H3 can inhibit the function of anti-tumor immune cells in many ways (31). And in the tumor tissues with high expression of B7-H3, there is a significant decrease in immune cells infiltration and the number of CD8+ T cells in the tumor microenvironment (32,33), further exacerbating poor prognosis. Further research on B7-H3's pro-tumor mechanisms, localization, function, and molecular pathways in cancer cells, will potentially expand the possibilities for targeted therapies aimed at B7-H3. Targeting HBEGF as a therapeutic target may potentially produce synergistic anti-tumor effects with B7-H3 targeted therapies.