Peer Review File

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Reviewer A

The paper titled "Bromodomain-containing protein 9 activates proliferation and epithelial-mesenchymal transition of colorectal cancer via the estrogen pathway in vivo and in vitro" is interesting. This study demonstrated that BRD9 could be an independent prognostic factor for CRC. Furthermore, the BRD9/estrogen pathway may contribute to the proliferation of CRC cells and EMT, suggesting that BRD9 may be a novel molecular target in the therapeutic treatment of CRC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) 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.

2) Figure 3 is missing scale bars. Please add on.

3) What are the relevant characteristics of the tumor microenvironment of colorectal cancer? What is the correlation between BRD9 and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

4) Colorectal cancer commonly metastasizes. The liver is the most frequent site of metastases and dominates the length of survival for this disease. What is the effect of BRD9 on colorectal cancer liver metastasis? It is recommended to include relevant content in the discussion.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Circ_0000799 promotes proliferation and invasion in colorectal cancer and epithelial-mesenchymal transition, J Gastrointest Oncol, PMID: 36636042". It is recommended to quote the articles.

6) There are many genes that regulate the colorectal cancer. Why did the author choose BRD9 for research? Please describe the reason.

7) What are the potential relationships between BRD9, epithelial-mesenchymal transition and cancer stem cells? How interaction of these processes may affect colorectal cancer progression, chemoresistance and ultimately recurrence? It is recommended to add relevant content.

Comment 1: 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 1: Thank you for your good suggestion, we have revised this manuscript according to your comment to suggest the knowledge gaps and limitations of prior study and the clinical significance of this study.

Changes in the text: Page 4, line 109-114.

Comment 2: Figure 3 is missing scale bars. Please add on.

Reply 2: Thank you for your good suggestion, we have revised Figure 3 according to your comment.

Changes in the text: Page 21, line 634.

Comment 3: What are the relevant characteristics of the tumor microenvironment of colorectal cancer? What is the correlation between BRD9 and the tumor microenvironment? What are the

possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply 3: Thank you for your good suggestion, we have revised this manuscript according to your comment. Colorectal immune regulation mainly involves colorectal mucosal epithelial cells, intestinal intraepithelial lymphocytes, and innate lymphocytes (including dendritic cells, intestinal T cells, and plasma cells). Moreover, dendritic cells, intestinal T cells, and plasma cells play an important role in the tumor microenvironment. In addition, BRD9 has been reported to be related to the tumor microenvironment, immune cell infiltration, and immune checkpoints, and was verified to be significantly associated with prognosis and tumor mutation burden in clear cell renal carcinoma(31). Thus, we mean to investigate the relationship between BRD9 and the immune microenvironment in colorectal cancer and develop new drug treatment methods in the future study.

Change in the text: see page 14 ,line 446-454.

Comment 4: Colorectal cancer commonly metastasizes. The liver is the most frequent site of metastases and dominates the length of survival for this disease. What is the effect of BRD9 on colorectal cancer liver metastasis? It is recommended to include relevant content in the discussion.

Reply 4: Thank you for your good suggestion, we have revised this manuscript according to your comment. Liver is the most frequent site of metastases and dominates the length of survival for colorectal cancer, while the study about the mechanism of colorectal cancer with liver metastasis of is rare, further studies using in vivo colorectal cancer models of liver metastasis will reveal the role of BRD9 in colorectal cancer with liver metastasis.

Change in the text: see page 15,line 467-473.

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Circ_0000799 promotes proliferation and invasion in colorectal cancer and epithelial-mesenchymal transition, J Gastrointest Oncol, PMID: 36636042”. It is recommended to quote the articles.

Reply 5:Thank you for your good suggestion, we have cited “Circ_0000799 promotes proliferation and invasion in colorectal cancer and epithelial-mesenchymal transition, J Gastrointest Oncol, PMID: 36636042” in the introduction part of our manuscript.

Change in the text: see page 15,line 460-462.

Comment 6: There are many genes that regulate the colorectal cancer. Why did the author choose BRD9 for research? Please describe the reason.

Reply 6: Thank you for your good suggestion, we have revised this manuscript according to your comment. In conclusion, these studies talked above suggested the potential role of BRD9

in cancer progression, however the literature has seldom study the function and mechanism of BRD9 in colorectal cancer.
Change in the text:see page 4, line 95-97.

Comment 7:What are the potential relationships between BRD9, epithelial-mesenchymal transition and cancer stem cells? How interaction of these processes may affect colorectal cancer progression, chemoresistance and ultimately recurrence? It is recommended to add relevant.
Reply 7:Thank you for your good suggestion, we have revised this manuscript according to your comment. Cancer stem cells (CSCs) and EMT promote the progression of CRC patients with liver metastasis. Moreover, EMT could induce the formation of CSCs and increase drug resistance. While, the specific molecular mechanisms and therapeutic resistance mechanisms between BRD9, EMT, and CSCs are still unclear. Activation of JAK/STAT3 signaling pathways induces the EMT, and results in increasing tumorigenicity and metastasis, promoting CSCs transformation, and drug resistance for CRC(37). The therapeutic strategies targeting the biomarkers of EMT are expected to overcome the drug resistance, cancer progression, and ultimately recurrence of CSCs in CRC. Our study has shown that BRD9 promotes the occurrence of EMT, thus we plan to explore the potential relationships between BRD9, EMT, and CRCs in the near future.
Change in the text:see page 15, line 474-484

Reviewer B

First, the title needs to indicate the prognostic role of BRD9.

Second, the abstract needs some revisions. The background did not indicate the potential clinical significance of this research focus and why the understanding on the mechanisms of BRD9 is important. The methods need to briefly describe the clinical sample, clinical factors, follow up procedures, and prognosis outcomes. The results need to briefly describe the clinical characteristics of the sample and quantify the findings by reporting outcome values, such as the expression levels, HR values, and P values. The conclusion needs to be more detailed for the clinical implications.

Third, the introduction of the main text needs to review what has been known on the prognostic biomarkers of CRC, have comments on their limitations and knowledge gaps, compare BRD with other known biomarkers to indicate the potential significance of the research focus on it, and indicate the clinical contribution of this study.

Fourth, in the methodology of the main text, please consider to have a flowchart to briefly describe the experimental procedures, and please consider to move many experimental details to a supplementary file since this part is too long. In statistics, the authors need to describe the procedures for ascertainment of the independent prognostic role of BRD9 and ensure $P<0.05$

is two-sided.

Comment 1:the title needs to indicate the prognostic role of BRD9.
Reply 1:Thank you for your good suggestion, we eager to revise our title according to your suggestion, however the prognostic role of BRD9 was discussed less compared to the colorectal cell function and cell signaling pathway of BRD9 in our manuscript. The following research we would like to explore more about the prognostic role of BRD9 in 524 colorecal cancer patients of our hospital.
Changes in the text:

Comment 2: The abstract needs some revisions. The background did not indicate the potential clinical significance of this research focus and why the understanding on the mechanisms of BRD9 is important. The methods need to briefly describe the clinical sample, clinical factors, follow up procedures, and prognosis outcomes. The results need to briefly describe the clinical characteristics of the sample and quantify the findings by reporting outcome values, such as the expression levels, HR values, and P values. The conclusion needs to be more detailed for the clinical implications.
Reply 2: Thank you for your good suggestion, we have revised this manuscript according to your comment. Follow up procedure and prognosis outcomes have been shown in the materials of the main text.
Change in the text: see page 1,line 27-34;page 2,line 38-44;page 2, line 51-54; page 2, line 61-62.

Comment 3: The introduction of the main text needs to review what has been known on the prognostic biomarkers of CRC, have comments on their limitations and knowledge gaps, compare BRD with other known biomarkers to indicate the potential significance of the research focus on it, and indicate the clinical contribution of this study.
Reply 3: Thank you for your good suggestion, we have revised this manuscript according to your comment. The literature have been reported that BRD9 might be an important biomarker in colon cancer, while their studies little investigate the clinical significance of BRD9 in colorectal cancer. Our study intended to explore the clinical significance of BRD9 in colorectal cancer. In addition, the relationship between BRD9 expression in CRC tissues and cell lines was examined. Moreover, we investigated the effects of BRD9 on the proliferation and EMT of CRC cells and discussed the potential molecular mechanisms. Finally, the influence of BRD9 knockdown on tumorigenesis was investigated in vivo.
Change in the text: see page 4,109-114.

Comment 4: In the methodology of the main text, please consider to have a flowchart to briefly describe the experimental procedures, and please consider to move many experimental details to a supplementary file since this part is too long. In statistics, the authors need to describe the procedures for ascertainment of the independent prognostic role of BRD9 and ensure $P<0.05$ is two-sided.

Reply 4: Thank you for your good suggestion, we have noticed the experimental details in the methodology is too long, and we will following your suggestion in the near future. Variables with a $P < 0.05$ in the univariate analysis were subjected to multivariate analysis using a Cox proportional hazards model to determine independent prognostic factors. The Kaplan-Meier method and the log-rank test were used for survival analysis. All tests were two sided, and P value <0.05 was considered statistically significant.

Changes in the text: see page 9, line 305-309.