Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Reviewer 1

kindly revise the language editing you need to discuss the clinical application of the used marker and its significance.

Response: Thanks for your sincere suggestion. We have reedited the language and supplemented the content of the potential clinical application of GMEB1 and its significance in the discussion part. Supplementary content is as follows:

The crucial role of the Hippo signaling pathway, with YAP/TAZ as the main effector proteins, in the development of hepatocellular carcinoma has been confirmed and highlighted by numerous studies[46], however, no inhibitors directly targeting core proteins of the Hippo signaling pathway have been successfully marketed, making direct intervention of the Hippo signaling pathway difficult and preventing patients with excessive Hippo activation from benefiting more from conventional therapies. For this reason, a number of studies have begun to attempt to interfere with the Hippo signaling pathway through the inhibition of indirectly regulated proteins. These include PFI-2, a highly selective inhibitor of SETD7 methyltransferase, which inhibits YAP nuclear translocation and Hippo pathway activation through direct interaction with SETD7[47], and Ki-16425, a competitive inhibitor of LPA1/2/3, which inhibits Hippo signaling by blocking LPA receptor-induced YAP/TAZ dephosphorylation[48]. The discovery of our study further enriches the range of target options for indirect intervention in the Hippo signaling pathway, thus providing new candidate proteins for inhibitor development.

In conclusion, our study revealed for the first time that GMEB1 is highly expressed in both HCC tissues and cells. Furthermore, we confirmed that YAP1 is a novel transcriptional target gene of GMEB1 and demonstrated that GMEB1/YAP1 regulatory axis has a key role in promoting HCC cell proliferation, migration and invasion and anti-apoptosis. As

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

this suggests that, on the one hand, GMEB1 can be a candidate molecular marker for accurate diagnosis of early-stage liver cancer; on the other hand, targeting GMEB1 may be a potential therapeutic approach to improve the efficiency of HCC treatment.

Reviewer 2

In this study, the authors reported that the highly expressed GMEB1 facilitates HCC progression by promoting the transcription of the YAP1 promoter region. 1. Abstract: "GMEB1 facilitated the malignant biological behaviors of HCC cells", the authors should make it clear what kind of malignant biological behaviors did GMEB1 promote in HCC, given that malignant behavior is a very broad term. 2. Introduction: the research background of HCC needs to be enriched with more statistics, such as the morbidity and mortality values. What is the link between GMEB1 and YAP1? It seems that the authors introduce the two molecules independently, without making the association clear. Additionally, the importance of GMEB1 in cancer development needs to be introduced more deeply. 3. Result: Firstly, the results at multiple levels indicate that GMEB1 is highly expressed in HCC tissues and cells compared to para-cancer tissues and normal human hepatocytes. Based on these findings, the authors thought that "GMEB1 plays a cancer-facilitating part in HCC progression." However, it does not allow us to conclude that GMEB1 play a pro-tumor part in HCC progression. The authors may consider a different statement to summarize these results. Secondly, "GMEB1 facilitates the malignancy of HCC cell line", this conclusion is too vague. Lastly, the authors should add the analysis of the experimental results in each part. 4. All the functional experiments were performed only in vitro assays. Therefore, the mechanistically importance of GMEB1 in HCC progression has not been fully validated. 5. The manuscript requires some wording and grammar revisions. For example: "Hepatocellular carcinoma (HCC) is a digestive system malignancy with high morbidity in clinical practice". "Cell counting kit-

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

8 method, Transwell and flow cytometry assays were utilized". "Some limitations exit in the present work".

Response: Thanks for your sincere suggestion. We have revised all the inappropriate statements and supplemented the content of related experiments as you mentioned and latest literature in the field. Details are as follows:

1. Abstract: "GMEB1 facilitated the malignant biological behaviors of HCC cells", the authors should make it clear what kind of malignant biological behaviors did GMEB1 promote in HCC, given that malignant behavior is a very broad term.

   We have changed the description as follows: GMEB1 facilitates HCC malignant proliferation and metastasis by promoting the transcription of the YAP1 promoter region.

2. Introduction: the research background of HCC needs to be enriched with more statistics, such as the morbidity and mortality values.

   We have supplemented the content as you would like to know in the manuscript. Specific information is as follows: Liver cancer is the third leading cause of cancer-related deaths worldwide, with more than 780,000 deaths due to liver cancer in 2018. Approximately 90% of liver cancer cases originate in hepatocytes and are referred to as hepatocellular carcinoma (HCC)[1]. HCC is the fifth most common cancer in males and the ninth most common cancer in females, with an estimated 500,000 and 200,000 new cases annually in the world, respectively. The pathogenesis of hepatocellular carcinoma is complicated by structural mutations in the proto-oncogene and the addition of exogenous pathogenic factors such as viruses, excessive alcohol consumption, obesity and aflatoxins, which have contributed to the development of HCC[2, 3].

3. Introduction: What is the link between GMEB1 and YAP1? It seems that the authors

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

introduce the two molecules independently, without making the association clear.

Unfortunately, there is no research or database analysis focused on the correlation between these two proteins. Therefore, after introducing the functions of the two proteins, we added such a paragraph to put forward our conjecture in order to build the linkage between the two, and paved the way for the following research logically. The added paragraph is as follows:

Could GMEB1, a transcription factor closely associated with tumor development, also promote the malignant progression of HCC by regulating the transcription of YAP1 and thus affecting the normal function of the Hippo signaling pathway? With this question in mind, we would like to confirm the potential regulatory relationship between GMEB1 and YAP1 in this study, thus providing a new intervention target for the Hippo signaling pathway, a key signaling pathway in HCC pathogenesis. To this end, we will firstly focus on examining the expression of GMEB1 in human HCC tissues and HCC cell lines, and clarifying the regulatory mechanism of the interaction between GMEB1 and YAP1 in promoting HCC progression in this study. We hope that our study will reveal the crucial role of GMEB1 in the development of HCC, explore the additional biological functions of GMEB1, and provide new therapeutic strategies for the clinical treatment of patients with HCC accompanied with YAP1 overexpression.

4. Introduction: Additionally, the importance of GMEB1 in cancer development needs to be introduced more deeply.

According to your suggestion, we conducted a literature survey of all the studies on the correlation between GMEB1 and tumor, and summarized all the research contents as follows:

It has been demonstrated that IL-2 can inhibit glucocorticoid-induced T-cell apoptosis by boosting GMEB1 expression and activating the PI3K/AKT pathway[10], which is a

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

preliminary indication of the anti-apoptotic function of GMEB1. Meanwhile, FOXL2 is an important transcription factor involved in the transcriptional regulation of several target genes, and GMEB1 was found to bind to FOXL2, whose interaction with FOXL2 could regulate the apoptotic process of cells[11]. Additionally, GMEB1 can also bind to pro-caspases and repress its activation and apoptosis [12, 13]. Given that tumor development cannot occur without uncontrolled cell proliferation and apoptotic escape, the anti-apoptotic effect of GMEB1 has prompted researchers to explore the relevance of GMEB1 dysregulation to tumorigenesis and development. For example, GMEB1 suppresses CASP8 activation via regulating CFLARL ubiquitination and degradation to repress the cellular apoptosis and thereby promoting malignant progression of non-small cell lung cancer[9]. Except from that, abnormally high GMEB1 expression in colorectal cancer (CRC) has been validated to be associated with increased lymph node invasion, poor TNM staging and reduced differentiation in CRC, and through mechanistic study, researchers further identified GMEB1 as a direct target gene of miR-320a, which negatively regulates GMEB1 expression, resulting in the inhibition of EMT, which is the prelude to CRC cell invasion and migration [14]. Additional bioinformatics studies have further shown that high expression of several transcription factors, including GMEB1, is a promising biomarker and/or therapeutic target for prostate cancer[15]. Thus, it becomes clear that GMEB1 can contribute to malignant progression in various tumor types through different mechanisms.

5. Result: Firstly, the results at multiple levels indicate that GMEB1 is highly expressed in HCC tissues and cells compared to para-cancer tissues and normal human hepatocytes. Based on these findings, the authors thought that "GMEB1 plays a cancer-facilitating part in HCC progression." However, it does not allow us to conclude that GMEB1 play a pro-tumor part in HCC progression. The authors may consider a different statement to summarize these results.

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

The conclusion in the section of " GMEB1 is highly expressed in HCC tissues and cells" has been restated and summarized as follows:

These results indicated that GMEB1 is highly expressed in HCC tissues and that upregulation of GMEB1 expression is strongly associated with an exacerbation of the malignant phenotype and poor prognosis. Also, this section provisionally suggested that the upregulation of GMEB1 may play a role in the malignant progression of hepatocellular carcinoma.

6. Result: Secondly, "GMEB1 facilitates the malignancy of HCC cell line", this conclusion is too vague.

   We have changed the vague conclusion into this:

   Taken together, we can easily infer that GMEB1 can enhance the proliferation and anti-apoptotic ability of tumor cells, thus promoting unlimited proliferation of tumor. In addition, the enhancement of the migration and invasion ability of tumor cells further increases the possibility of tumor metastasis.

7. Result: Lastly, the authors should add the analysis of the experimental results in each part.

   We have rewritten the whole result part and added the analysis of the experimental results in each section.

8. Result: All the functional experiments were performed only in vitro assays. Therefore, the mechanistically importance of GMEB1 in HCC progression has not been fully validated.

   According to your opinion, we added the experiment of subcutaneous tumor transplantation in nude mice and tumor metastasis model constructed by injection of tumor cells via tail vein to confirm the effect of GMEB1 in promoting tumor malignant proliferation and increasing metastatic potential in vivo. The data are presented in FIG. 5 and the results are described and analyzed as follows:

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

To further validate this effect, we then constructed sh-GMEB1, oe-YAP1 and sh-GMEB1+oeYAP1 stable expression cell lines by lentiviral infection and investigated the effect of the GMEB1/YAP1 regulatory loop on tumor proliferation by Cell-derived xenografts (CDX) model in nude mice. The results revealed that sh-GMEB1 remarkably slowed down the proliferation rate of HepG2-derived xenografts, whereas overexpression of YAP1 significantly enhanced the proliferation ability of tumors compared with the oe-NC group. More interestingly, silencing GMEB1 followed by overexpression of YAP1 markedly reversed the proliferation inhibitory effect of silencing GMEB1 (Figure 5A-B). The above data suggested that the GMEB1/YAP1-regulated signaling axis can effectively increase the level of malignant proliferation of tumor. Subsequently, we examined whether the number of HCC metastasis foci forming in the lung would be altered by tail vein injection of the above cells. As we can see, silencing of GMEB1 significantly reduced the number of metastatic foci, whereas overexpression of YAP1 did the opposite. Furthermore, silencing of GMEB1 followed by overexpression of YAP1 also reversed the inhibitory effect of GMEB1 silencing on the metastatic ability, with no significant difference from the control group (Figure 5C-D).

9. The manuscript requires some wording and grammar revisions. For example: “Hepatocellular carcinoma (HCC) is a digestive system malignancy with high morbidity in clinical practice”. “Cell counting kit-8 method, Transwell and flow cytometry assays were utilized”. “Some limitations exit in the present work”.

   We have revised the grammar through the professional language polishing. The listed inappropriateness has been rewritten as follows:

   1. HCC is one of the most common types of clinical malignancies in the digestive system.
   2. Cell counting kit-8 assay, transwell assay and flow cytometry were utilized to

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

examine HCC cell proliferation, migration, invasion and apoptosis, respectively.

3. However, our study also has some limitations. Firstly, we only used the CDX animal model to verify the regulation of GMEB1 on the malignant progression of HCC; we lacked samples from clinical sources for further corroboration. In addition, we lacked follow-up studies on patients so as to further corroborate the effect of GMEB1 on the prognosis of hepatocellular carcinoma. In subsequent studies, we will continue to refine our findings in the follow-up study and continue to obtain more reliable experimental conclusions.