

Peer Review File

Article Information: <https://dx.doi.org/10.21037/jgo-23-447>

Reviewer A:

Abstract:

- Line 24: "...in human cancers and participated in multiple biological behaviors". It's not clear which biological behaviors are being referred to. Could the authors specify which biological processes or behaviors they mean?

Response: In page 1, line 23-24, the specific biological behaviors have been added in the manuscript.

- Improvements in organization and clarity within the methods section would greatly enhance comprehension. This could be achieved, for example, by categorizing experimental procedures based on their type, such as techniques pertaining to patient samples, those related to cell culture studies, and molecular biology methodologies.

Response: In page 1-2, line 26-37, the organization in the methods section has been reorganized based on the modification suggestions.

Introduction:

- The statement "Hepatocellular carcinoma (HCC) is a common malignant tumor in humans with high incidence rate and mortality in China" could be more effectively contextualized by providing global incidence and mortality rates as well. This would better highlight the global relevance of the study.

Response: In page 2, line 53-56, we have re-described the latest situation of liver cancer in global cancer.

- Lines 52-55: "Improvements of surgical skills and the assistance ... in the clinical therapy of HCC". The sentence is grammatically incorrect and needs a revision for clarity. The sentence is missing a connecting element to link the improvements to the resulting progress.

Response: In page 2, line 59-60, we have modified the description.

- The authors could further clarify the current understanding of HCC, including its major risk factors, the most commonly affected populations, and the typical prognosis, to better set the stage for the research.

Response: In page 2-3, line 56-59, we have added the risk factors of HCC in the manuscript. In page 3, line 64-65, we have modified the prognostic information of HCC.

- The authors should explicitly stating their hypothesis or expected findings based on existing knowledge of HMGB2 in HCC and other cancers.

Response: In page 3, line 78-81, the expression pattern and biological behaviors of HMGB2 in various human tumors, including colorectal cancer, liver cancer, and glioma, have been described in the manuscript. We supplemented our hypothesis in page 3, line 83-85, and expected findings in page 3, line 88-89.

- The phrasing "unambiguous function of HMGB2 in HCC is largely unknown" could be rephrased for clarity. It is slightly contradictory as it is currently written; perhaps the authors meant "definitive role" or "specific function".

Response: In page 3, line 81-82, we have modified such description.

Materials and methods:

- The text has some grammatical issues and awkward phrasing, which may interfere with the reader's understanding. For example, in line 133, the phrase "HCC tissues and cells was dissociated" should be "HCC tissues and cells were dissociated". Careful editing to correct such errors would improve the readability and clarity of this section.

Response: Thank you for your helpful suggestions. We have carefully edited the whole manuscript and modified such inaccurate descriptions to improve reader's understanding.

- In the section on "Patients and specimens": This section needs some further explanations and clearer transitions to improve the flow of the text.

Response: In page 4, line 99-117, we have modified "Patients and specimens" section.

- In the section on "Cell culture": The relationship between the human HCC cell lines and the THLE-2 line is not clearly stated. The use of "they were" in the second sentence may confuse readers as to what "they" refers to - it could be interpreted as the HCC cells when it is likely meant to refer to the THLE-2 cells.

Response: In page 5, line 119-120, we have corrected the description.

- In the section on "Cell viability, migration, and invasion assays": It might be helpful to provide a bit more detail on how they were conducted.

Response: In page 5-6, line 133-152, we have rewritten the "Cell viability, migration, and invasion assays" section.

- In the section on "Immunohistochemistry": "Following antibodies were used" should be changed to "The following antibodies were used", to improve the clarity and flow of the text.

Response: In page 6, line 155, we have corrected the description.

- In the section on "Western blot analysis": The reviewer suggests changing: "The extracted protein HCC samples and HCC cells were electrophoresed and visualized..." to "Proteins were extracted from HCC samples and cells, then separated and visualized..." for clarity. Furthermore, adding a subject ("The following antibodies") to the sentence that lists the antibodies used for clarity.

Response: In page 7, line 179-183, we have corrected the description.

Statistical analysis:

- The reviewer suggests stating the specific tests used for each analysis. For example, which specific comparisons used a Chi-squared test, Fisher's exact test, or Student-t test?

Response: In page 8, line 207-215, we have modified the "Statistical analysis" section.

Results:

- This section could benefit from clearer, more precise language in several areas. For example, "As a nucleoprotein, HMGB2 can regulate various biological processes by binding transcription factors, such as p53, p73, lymphoid enhancer-binding factor 1 (LEF1) and Runt-related transcription factor 2 (Runx2)" could be rewritten as, "HMGB2, a nucleoprotein, can regulate various biological processes by interacting with transcription factors such as p53, p73, LEF1, and Runx2."

Response: In page 9, line 243-247, the inaccurate description has been corrected.

- In the section on "Nucleoprotein HMGB2 is overexpressed in HCC": The phrase "preponderantly located in nucleus and cytoplasm" could be better phrased as "predominantly located in the nucleus and cytoplasm."

Response: In page 8, line 222-223, the inaccurate description has been corrected.

- It would be beneficial to provide more background information or citations on why MHCC97H and LM3 HCC cell lines were chosen as the cell models.

Response: In page 8-9, line 227-232, we have supplemented the relevant reasons for selecting these two cells as model cells.

- The statement "Our findings suggested ZEB1 and vimentin protein expression were higher in HCC samples accompanied with an increase of HMGB2 and mRNA and protein" could be clarified. It's not clear whether the authors are suggesting that the increase in HMGB2 expression causes the increase in ZEB1 and vimentin, or if they're merely co-occurring.

Response: Based on the high expression of HMGB2 in HCC cells with high metastatic potential, we are interested in exploring its correlation with other proteins and transcription factors related to metastasis. ZEB1 and Vimentin are known and recognized important factors involved in tumor metastasis. We have supplemented the relevant reasons in the manuscript (In page 9, line 234-238), and the corresponding references have also been supplemented.

- In the section on "HMGB2 deprivation restricts the proliferation migration and invasion ability of HCC cells in vitro": The authors might want to clarify the statement "that the above findings suggested decreased expression of HMGB2 could restrict the malignant proliferation, migration and invasion of HCC cells." The use of "that" in the beginning of the sentence makes it unclear and potentially grammatically incorrect. A better phrasing could be: "The above findings suggest that decreased expression of HMGB2 could restrict the malignant proliferation, migration, and invasion of HCC cells."

Response: In page 10, line 263-267, the inaccurate description has been corrected.

- The authors should ensure that the titles of the figures and tables are clear and descriptive, defining all abbreviations in the legends of the figures and tables and the statistical methods used in the analysis should be mentioned in the legend

Response: 1. Student-t test were employed for comparison between groups (Figure 1-4, Figure S2-3); 2. Chi-squared tests and Fisher's exact tests were employed to analyzed the clinical

information (Table 1); 3. Kaplan-Meier method were employed to further analyze prognosis and survival information of HCC patients (Figure 5); 4. Multivariate Cox proportional hazards model were employed to further analyze clinical data (Table 2). These modifications have been added in Figure Legends section and tables. In page 19-21, line 527-591.

Discussion:

- The authors have mentioned that the overexpression of HMGB2 was found particularly in advanced HCC samples. While they speculate that HMGB2 might be a part of the malignant development of HCC, they could expand this part by discussing possible mechanisms by which HMGB2 might promote the progression from early to advanced HCC.

Response: In page 13, line 358-365, the discussion of possible mechanism of HMGB2 highly expressed in advanced HCC has been supplemented.

- The authors suggest that HMGB2 might be a potential target for drug development. While this is a fascinating suggestion, it would be helpful if they could elaborate on this point and provide some examples or strategies on how this could be achieved.

Response: In page 13, line 372-375, the future research strategies for drug development by using HMGB2 has been supplemented.

- A more detailed explanation of the HMGB2-ZEB1-vimentin axis would be useful. While the authors briefly mention this axis and its possible role in HCC, more background information or a more detailed description would be beneficial for readers not familiar with this specific topic.

Response: In page 12-13, line 335-348, the background information of Vimentin and ZEB1 has been supplemented.

- The reviewer suggests adding a section discussing the limitations of their study. For example, it seems that their work was largely conducted in cell lines and tissues, so they might acknowledge that further work (potentially in animal models or larger cohorts of patients) will be needed to fully understand the relevance of their findings.

Response: In page 14, line 380-384, the limitations of their study has been supplemented.

- In the future work section, the authors might provide more specific examples of what these future experiments might look like. For example, what kind of experiments could they conduct to further study the interaction between HMGB2, ZEB1, and vimentin?

Response: In page 13-14, line 372-375, line 380-384, line 390-395, the future research directions and possible research methods have been supplemented in the manuscript.

Conclusion:

- The conclusion would benefit from an explicit connection between the research findings and their implications for diagnosing or treating HCC. The novelty of the authors' results should be accentuated, highlighting how their work differs from or expands upon previous studies. The unique contribution this paper makes to our understanding of HCC needs to be clearer in order to underscore its relevance and significance in the field.

Response: In page 14, line 390-395, we have rewritten the "Conclusion" section.

Reviewer B:

1. Cell map must describe magnification and the staining method in the Figure Legend (or there is “Scale bar” on the image). Please revise it for all relative figures. (fig 1, 3, S1, S2)

Response: The scale bar has been described in the Figures (Fig1, 3, S1 and S2) and each Figure Legend.

2. It seems that the following affiliation is different from the authors’ affiliation. Please clarify.

Response: Eastern Hepatobiliary Surgery Hospital is the Third Affiliated Hospital of Naval Military Medical University.

90 **2.1 Ethics statement.**

91 Collection and use of clinical tissues, relevant pathological and follow-up data were
92 approved by Biomedical Ethics Committee of The Third Affiliated Hospital of Navy
93 Military Medical University (Approval Number: EHBHKY2020-02-003; Shanghai,
94 China). Written informed consent was received from each patient prior to the surgery.
95 ~~The study was conducted in accordance with the Declaration of Helsinki (as revised~~
96 ~~in 2013).~~

97 **2.2 Patients and specimens.**

98 HCC tissues (n=62) were obtained from patients who received radical local
99 hepatectomy in the third affiliated hospital of navy military medical university from
100 February 2013 to January 2014. All cases were confirmed diagnosis of primary HCC
101 by imaging, serological diagnosis and histopathology. All patients did not receive any

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10 Military Medical University, Shanghai 200438, P.R. China.

3. pls add citations for the following statement.

279 differentiation of different cells. In recent years, accumulating studies have shown that
280 abnormal elevation of HMGB2 can play an important role as a biomarker of tumor
281 progression, metastasis, differentiation and it can also predict the prognosis of patients.
282 However, up to now, the explicit function of HMGB2 in HCC is still a mystery.

Response: The citations have been added.