ESPS Manuscript NO: 79276

Title: Recent progress in molecular mechanisms of postoperative recurrence and metastasis of hepatocellular carcinoma

Author: Zhao-Shan Niu, Wen-Hong Wang and Xiao-Jun Niu

Name of Journal: *World Journal of Gastroenterology*

Dear Editor and Reviewers,

Thank you for carefully reviewing our manuscript entitled " Recent progress in molecular mechanisms of postoperative recurrence and metastasis of hepatocellular carcinoma" for possible publication in the World Journal of Gastroenterology as a review. We appreciate the comments and suggestions from the editor and reviewers. We have revised the manuscript, highlighting our revisions in red, and have attached point-by-point responses detailing how we have revised the manuscript according to the reviewers' comments below.

Yours sincerely,

Zhao-Shan Niu (Corresponding Author)

Reviewer #1

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors:

This review is written very well. It covers all relevant molecular mechanism

involved in recurrence and metastasis of HCC.

Response: The authors greatly appreciate the reviewer's positive comment. The language in this paper has been re-edited and polished by American Journal Experts(AJE).

Reviewer #2

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

The manuscript is well organized. While the topic is timely, the manuscript would benefit from substantial revisions throughout to aid with clarity.

Response: The authors greatly appreciate the reviewer's positive comment. Our deepest gratitude goes to you for your careful work and thoughtful suggestions that have helped improve this paper substantially.

In addition, the language in this paper has been re-edited and polished by American Journal Experts(AJE).

1) What do you mean by "intrahepatic distant metastasis", as "HCC metastasis can be roughly divided into intrahepatic and extrahepatic (distant) metastasis.", on Page 3.

Response: Thank you for this reminder. We have corrected this mistake. The phrase "and intrahepatic distant metastasis and postoperative recurrence represent the major causes of its high mortality" in the original manuscript has been changed to "with postoperative metastasis and recurrence causing much of its high mortality" in the revised manuscript.

2) Page 4: "Of these processes, the activation of oncogenes and the

inactivation of tumor suppressor genes can lead to uncontrolled growth of cancer cells and thus have the potential to metastasize." This sentence is confusing as written, as the subject is misused for "have the potential to metastasize".

Response: We are very sorry that this sentence is confusing, and we have revised it according to the reviewer's comments. The sentence "Of these processes, the activation of oncogenes and the inactivation of tumor suppressor genes can lead to uncontrolled growth of cancer cells and thus have the potential to metastasize." in the original manuscript has been changed to "The activation of oncogenes and the inactivation of tumor suppressor genes can lead to uncontrolled growth of cancer cells, enabling invasion and metastasis." in the revised manuscript.

3) Page 5: "OPN is a glycoprotein with secretory calcium-binding phosphorylation", and "OPN can secrete stromal cells and chemokines", these sentences were confusing.

Response: We are very sorry that these sentences were confusing and we have revised them according to the reviewer's comments. The sentences "OPN is a glycoprotein with multiple biological activities and a secretory calcium-binding phosphoprotein whose encoding gene is located on chromosome 4q13. OPN has diverse functions and can secrete stromal cells and chemokines, among other factors, to help regulate cell functions, regulate signal transduction by binding integrins, and facilitate the formation and progression of tumors [10]." in the original manuscript have been changed to "OPN is a glycoprotein and a secretory calcium-binding phosphoprotein with multiple biological activities encoded on chromosome 4q13. OPN can exert diverse functions by interacting with integrins or CD44 receptors to regulate cell adhesion and cell chemotaxis, regulate signal transduction, and facilitate tumor formation and progression [10]" in the revised manuscript.

4) Page 6: "Therefore, S100A9 exhibits poor stability; is prone to deletion, translocation and other gene mutations; and is connected to the growth, differentiation and metastasis potential of various malignancies", this sentence has grammar problems, as a subject is needed after each semicolon. In addition, what do you mean by saying "S100A9 is prone to other gene mutations"?

Response: We apologize for the language problems, and we have revised the text to address the reviewer's comments. The sentence "Therefore, S100A9 exhibits poor stability; is prone to deletion, translocation and other gene mutations; and is connected to the growth, differentiation and metastasis potential of various malignancies." in the original manuscript has been changed to "Therefore, S100A9 exhibits poor stability and is prone to chromosomal deletion and translocation, which are connected to the growth, differentiation and metastasis potential of various malignancies" in the revised manuscript.

5) Page 6: define "MDSCs" when it appears for the first time.

Response: We are grateful for the suggestion. As suggested by the reviewer, we have defined "MDSCs" at its first appearance. The sentence "S100A9 in HCC tissues induces the migration and aggregation of bone marrow-derived myeloid-derived suppressor cells (MDSCs)" in the original manuscript has been changed to "S100A9 in HCC tissues induces the migration and aggregation of bone marrow-derived myeloid-derived suppressor cells (MDSCs), a heterogeneous population composed of immature myeloid cells with immunosuppressive properties," in the revised manuscript.

6) Page 10: confirm that reference 66 was correctly cited, as "tumor resection" was not mentioned in this paper.

Response: We are extremely grateful to the reviewer for pointing out this

problem. The original reference 66 "66 **Mizukoshi E,** Kaneko S. Immune cell therapy for hepatocellular carcinoma. *J Hematol Oncol* 2019; **12**:52 [PMID: 31142330 DOI: 10.1186/s13045-019-0742-5]" in the original manuscript has been replaced with reference 63 "63 **Chen X,** Chi H, Zhao X, Pan R, Wei Y, Han Y. Role of Exosomes in Immune Microenvironment of Hepatocellular Carcinoma. *J Oncol* 2022; **2022**:2521025 [PMID: 35126514 DOI: 10.1155/2022/2521025]" in the revised manuscript.

Moreover, the "Currently, considerable evidence indicates that the disturbance of the TIME caused by tumor resection is key to promoting immune evasion, metastasis and recurrence in HCC [66]." in the original manuscript has been changed to "Currently, considerable evidence indicates that the TIME is key to promoting immune evasion, metastasis and recurrence in HCC [63]." in the revised manuscript.

7) Page 20: "HSCs play important roles in promoting HCC invasion and metastasis by multiple mechanisms." This paragraph clarifies the action of HSCs after activated. But it may be more important to focus on the molecular mechanisms that activated HSCs, for possible intervention of HCC recurrence and metastasis. The authors should address these mechanisms in the manuscript.

Response: We deeply appreciate the reviewer's suggestion. According to the reviewer's comment, we have addressed the molecular mechanisms of HSC activation in the first paragraph of the section on HSCs in the revised manuscript as follows:

"HSC activation is the central link in hepatic fibrosis caused by various etiologies, and activated HSCs play an important role in HCC metastasis and recurrence. Therefore, elucidating the molecular mechanisms of HSC activation is of great significance in preventing or reversing hepatic fibrosis and in preventing postoperative HCC recurrence and metastasis. The mechanisms of HSC activation are complicated. In addition to cytokines,

transcription factors and oxidative stress can activate HSCs, and ncRNAs and exosomes have also been found to be involved in HSC activation in recent years. In brief, the mechanisms of HSC activation are as follows. (1) Cytokines participate in HSC activation. When the liver is injured, hepatocytes, Kupffer cells, sinusoidal endothelial cells, macrophages and platelets all secrete cytokines that play decisive roles in HSC activation, such as TGF-β and PDGF. The mechanisms of action by which cytokines activate HSCs are quite complex. Rather than acting in isolation, cytokines form a network through autocrine and paracrine interactions, thereby activating HSCs. TGF-β can activate HSCs by binding to specific receptors of HSCs and activating both SMAD-dependent and non-SMAD pathways [178]. TGF-β can also activate HSCs through HAS2, TLR4he and Notch1 [179]. PDGF is mainly produced by platelets, Kupffer cells and sinusoid endothelial cells in the liver. After PDGF binds to the receptor on the membrane of HSCs, it promotes HSC proliferation and activation mainly through PI3K/Akt, JAK/STAT, and MAPK [180-182]. (2) Transcription factors participate in HSC activation. Transcription factors related to HSC activation mainly include nuclear factor-κB (NF-κB) and peroxisome proliferator-activated receptor γ (PPAR-γ). NF-κB is an important intracellular mediator of the liver inflammatory response. NF-κB activation can promote HSC activation by promoting the generation of proinflammatory factors such as interleukin-8 (IL-8), interleukin-1β (IL-1β), TNF-α and interleukin-6 (IL-6) [183,184]. PPAR-γ is a class of ligand-activated nuclear receptor transcription factors that are members of the nuclear hormone receptor superfamily. PPAR-γ exerts anti-inflammatory effects by inhibiting the transcription of inflammatory cytokines and has a regulatory effect on maintaining HSC quiescence and inhibiting HSC activation. It has been shown that PPAR-γ inhibits HSC activation mainly by inhibiting the activity of the TGF-β1/Smad pathway or by downregulating the expression of potent fibrogenic factors such as PDGF, connective tissue growth factor (CTGF), and TGF-β1 [185,186]. (3) Oxidative

stress is an important factor in HSC activation. When oxidative stress occurs, excessive production of ROS *in vivo* not only directly damages hepatocytes but also activates various signaling pathways, such as nuclear factor-related factor-2 (Nrf2), TGF-β, NF-κB and other related cytokines, signaling molecules and their downstream signaling pathways, that further aggravate liver tissue injury and activate the production of various inflammatory factors. This process further stimulates the continuous activation of NF-kB and regulates apoptosis-related proteins, IL-1β, TNF-α and IL-6, which play important roles in promoting HSC activation and proliferation [187,188]. (4) NcRNAs are also involved in HSC activation. NcRNAs include microRNAs (miRNAs), long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), which can regulate HSC activation through a variety of mechanisms. Among them, oncogenic ncRNAs facilitate HSC activation. For example, microRNA-503 overexpression can enhance HSC activation by activating the TGF-β/Smad pathway [189]; the upregulation of lncRNA SNHG7 promotes HSC activation by sponging miR-29b, which enhances DNA methyltransferase 3A (DNMT3A) expression [190]; and the upregulation of circUbe2k facilitates HSC activation by sponging miR-149-5p, which enhances TGF-β2 expression [191]. In contrast, tumor suppressive ncRNAs inhibit HSC activation. For example, overexpression of miR-489-3p and miR-122-5p suppresses HSC activation by restraining the uneven regulation of the jagged canonical Notch ligand 1 (JAG1)/notch homolog protein 3 (NOTCH3) signaling pathway [192]; upregulation of lncRNA Meg8 inhibits HSC activation by suppressing the Notch pathway [193]; and increased expression of hsa_circ_0070963 suppresses HSC activation by enhancing LEM domain containing 3 (LEMD3) expression by sponging miR-223-3p [194]. (5) Exosomes regulate HSC activation through various mechanisms. For example, high expression of exosomal miR-192 derived from HCV-infected hepatocytes can stimulate TGF-β1 expression and thus induce HSC activation [195]. In contrast, NK-cell-derived exosomes can inhibit TGF-β1-induced HSC

activation [196].”

178 **Nair B,** Nath LR. Inevitable role of TGF-β1 in progression of nonalcoholic fatty liver disease. *J Recept Signal Transduct Res* 2020; **40**:195-200 [PMID: 32054379 DOI: 10.1080/10799893.2020.1726952]

179 **Yang YM,** Noureddin M, Liu C, Ohashi K, Kim SY, Ramnath D, Powell EE, Sweet MJ, Roh YS, Hsin IF, Deng N, Liu Z, Liang J, Mena E, Shouhed D, Schwabe RF, Jiang D, Lu SC, Noble PW, Seki E. Hyaluronan synthase 2-mediated hyaluronan production mediates Notch1 activation and liver fibrosis. *Sci Transl Med* 2019;**11**: eaat9284 [PMID: 31189722 DOI: 10.1126/scitranslmed. aat9284]

180 **Zhang SL,** Ma L, Zhao J, You SP, Ma XT, Ye XY, Liu T. The Phenylethanol Glycoside Liposome Inhibits PDGF-Induced HSC Activation via Regulation of the FAK/PI3K/Akt Signaling Pathway. *Molecules* 2019; **24**:3282 [PMID: 31505837 DOI: 10.3390/molecules24183282]

181 **Li Y,** Xia JY, Chen W, Deng CL. [Effects of Ling Qi Juan Gan capsule drug-containing serum on PDGF-induced proliferation and JAK/STAT signaling of HSC-T6 cells]. *Zhonghua Gan Zang Bing Za Zhi* 2013; **21**:663-667 [PMID: 24160340 DOI: 10.3760/cma.j.issn.1007-3418.2013.09.005]

182 **Cai S,** Wu L, Yuan S, Liu G, Wang Y, Fang L, Xu D. Carvacrol alleviates liver fibrosis by inhibiting TRPM7 and modulating the MAPK signaling pathway. *Eur J Pharmacol* 2021; **898**:173982 [PMID: 33647257 DOI: 10.1016/j.ejphar.2021.173982]

183 **Wang A,** Zhang F, Xu H, Xu M, Cao Y, Wang C, Xu Y, Su M, Zhang M, Zhuge Y. TWEAK/Fn14 promotes pro-inflammatory cytokine secretion in hepatic stellate cells via NF-κB/STAT3 pathways. *Mol Immunol* 2017; **87**:67-75 [PMID: 28411440 DOI: 10.1016/j.molimm.2017.04.003]

184 **Wang H,** Che J, Cui K, Zhuang W, Li H, Sun J, Chen J, Wang C. Schisantherin A ameliorates liver fibrosis through TGF-β1mediated activation

of TAK1/MAPK and NF-κB pathways in vitro and in vivo. *Phytomedicine* 2021; **88**:153609 [PMID: 34126414 DOI: 10.1016/j.phymed.2021.153609]

185 **Ni XX,** Li XY, Wang Q, Hua J. Regulation of peroxisome proliferator-activated receptor-gamma activity affects the hepatic stellate cell activation and the progression of NASH via TGF-β1/Smad signaling pathway. *J Physiol Biochem* 2021; **77**:35-45 [PMID: 33188625 DOI: 10.1007/s13105-020-00777-7]

186 **Alatas FS,** Matsuura T, Pudjiadi AH, Wijaya S, Taguchi T. Peroxisome Proliferator-Activated Receptor Gamma Agonist Attenuates Liver Fibrosis by Several Fibrogenic Pathways in an Animal Model of Cholestatic Fibrosis. *Pediatr Gastroenterol Hepatol Nutr* 2020; **23**:346-355 [PMID: 32704495 DOI: 10.5223/pghn.2020.23.4.346]

187 **Ramos-Tovar E,** Muriel P. Molecular Mechanisms That Link Oxidative Stress, Inflammation, and Fibrosis in the Liver. *Antioxidants (Basel)* 2020; **9**:1279 [PMID: 33333846 DOI: 10.3390/antiox9121279]

188 **Ge C,** Tan J, Lou D, Zhu L, Zhong Z, Dai X, Sun Y, Kuang Q, Zhao J, Wang L, Liu J, Wang B, Xu M. Mulberrin confers protection against hepatic fibrosis by Trim31/Nrf2 signaling. *Redox Biol* 2022; **51**:102274 [PMID: 35240537 DOI: 10.1016/j.redox.2022.102274]

189 **Xie X,** Dou CY, Zhou Y, Zhou Q, Tang HB. MicroRNA-503 Targets Mothers Against Decapentaplegic Homolog 7 Enhancing Hepatic Stellate Cell Activation and Hepatic Fibrosis. *Dig Dis Sci* 2021; **66**:1928-1939 [PMID: 32648079 DOI: 10.1007/s10620-020-06460-7]

190 **Xie Z,** Wu Y, Liu S, Lai Y, Tang S. LncRNA-SNHG7/miR-29b/DNMT3A axis affects activation, autophagy and proliferation of hepatic stellate cells in liver fibrosis. *Clin Res Hepatol Gastroenterol* 2021; **45**:101469 [PMID: 32893175 DOI: 10.1016/j.clinre.2020.05.017]

191 **Zhu S,** Chen X, Wang JN, Xu JJ, Wang A, Li JJ, Wu S, Wu YY, Li XF, Huang C, Li J. Circular RNA circUbe2k promotes hepatic fibrosis via

sponging miR-149-5p/TGF-β2 axis. *FASEB J* 2021;35: e21622 [PMID: 33982351 DOI: 10.1096/fj.202002738R]

192 **Li J,** Dong S, Ye M, Peng G, Luo J, Wang C, Wang J, Zhao Q, Chang Y, Wang H. MicroRNA-489-3p Represses Hepatic Stellate Cells Activation by Negatively Regulating the JAG1/Notch3 Signaling Pathway. *Dig Dis Sci* 2021; **66**:143-150 [PMID: 32144602 DOI: 10.1007/s10620-020-06174-w]

193 **Chen T,** Lin H, Chen X, Li G, Zhao Y, Zheng L, Shi Z, Zhang K, Hong W, Han T. LncRNA Meg8 suppresses activation of hepatic stellate cells and epithelial-mesenchymal transition of hepatocytes via the Notch pathway. *Biochem Biophys Res Commun* 2020; **521**:921-927 [PMID: 31711641 DOI: 10.1016/j.bbrc.2019.11.015]

194 **Ji D,** Chen GF, Wang JC, Ji SH, Wu XW, Lu XJ, Chen JL, Li JT. Hsa_circ_0070963 inhibits liver fibrosis via regulation of miR-223-3p and LEMD3. *Aging (Albany NY)* 2020; **12**:1643-1655 [PMID: 32003753 DOI: 10.18632/aging.102705]

195 **Kim JH,** Lee CH, Lee SW. Exosomal Transmission of MicroRNA from HCV Replicating Cells Stimulates Transdifferentiation in Hepatic Stellate Cells. *Mol Ther Nucleic Acids* 2019; **14**:483-497 [PMID: 30753992 DOI: 10.1016/j.omtn.2019.01.006]

196 **Wang L,** Wang Y, Quan J. Exosomes derived from natural killer cells inhibit hepatic stellate cell activation and liver fibrosis. *Hum Cell* 2020; **33**:582-589 [PMID: 32449114 DOI: 10.1007/s13577-020-00371-5]

8) Page 24: "ECs can be directly and selectively targeted to inhibit or kill TECs and inhibit or prevent HCC metastasis and recurrence.", this sentence was confusing as written.

Response: We apologize for this confusing sentence in the original manuscript, and we have revised the text according to the reviewer's comments. The sentence "ECs can be directly and selectively targeted to inhibit or kill TECs

and inhibit or prevent HCC metastasis and recurrence." in the original manuscript has been changed to "TECs can be directly and selectively inhibited or killed to suppress or prevent HCC metastasis and recurrence" in the revised manuscript.

9) Page 25: "Changes in adhesion in tumor cell-ECM and HCC metastasis", do you mean "Adhesion enhancement in tumor cell-ECM promotes HCC metastasis"?

Response: We agree with the reviewer's point. The phrase" Changes in adhesion in tumor cell-ECM and HCC metastasis" in the original manuscript has been changed to "Tumor cell–ECM adhesion enhancement in HCC metastasis" in the revised manuscript.

10) Page 30: UPA is the abbreviation for Urokinase-like Plasminogen Activator, but not "plasminogen activator". In addition, if you mean "Urokinase-like Plasminogen Activator", three abbreviations UPA, u-PA and uPA (page 31) are used in the text, choose one of them.

Response: We deeply appreciate the reviewer's suggestion. According to this advice, we have made the three abbreviations we used for "urokinase-type plasminogen activator" (UPA, u-PA and uPA) consistent by using "uPA" in the revised manuscript.

11) Page 30-31: "A prerequisite for HCC metastasis and recurrence after surgery is the degradation of the ECM and BM by a series of proteolytic enzymes. During HCC aggressiveness and metastasis, only when HCC cells disrupt the dynamic balance of the ECM and penetrate the BM can they invade the surrounding tissues and cause HCC diffusion and metastasis. This process subsequently depends on two important enzymes. One is uPA secreted by tumor cells, which can promote HCC invasion and metastasis by degrading the ECM and penetrating the BM [307,308]. The other enzymes are

MMPs that are correlated with HCC metastasis and recurrence, and the roles of MMP-2 and MMP-9 have been confirmed in many studies [309-311].", this paragraph can be deleted, as the content has been expressed in the previous two paragraphs.

Response: We agree with the reviewer's suggestion. Accordingly, this paragraph has been deleted in the revised manuscript.

12) Page 31: "In short, specific inhibitors targeting MMP-2, MMP-9 and uPA inhibit the activities of MMP-2, MMP-9 and uPA and further inhibit HCC growth, invasion and metastasis.", this sentence is not appropriate, because there is no mention on the pharmacological inhibitors of these proteins before.

Response: We are extremely grateful to the reviewer for pointing out this problem. We have deleted this sentence in the revised manuscript.

13) Page 32: "In addition, IFN-γ can inhibit activated T helper type-1 (Th1) lymphocytes and the generation of IFN-γ and IL-2 by increasing the expression of Fc receptors on the membrane of macrophages. Thus, the levels of the inhibitory factor of macrophage factor synthesis are increased, which inhibits the immune response and is tightly associated with HCC development [315].", these sentences are confusing. First, can IFN-γ inhibit the generation of itself in macrophage, and is it mentioned in ref 315? Second, is it dependent on the inhibition of activated T helper type-1 (Th1) lymphocytes by IFN-γ, and is it mentioned in ref 315? Third, ref 315 is negative on IFN-γ effect of inhibiting HCC occurrence, but ref 314 and 316 are positive. Discuss ref 315 at the end. Last, by saying "of macrophage factor synthesis", is it better for "on synthesis of macrophage factors", and what do you mean by "the inhibitory factor"?

Response: We are sorry that these sentences were confusing. Because deleting them did not affect the completeness and meaning of the content, we have deleted these sentences in the revised manuscript.

14) Page 32: "One probable reason is that a low level of IFN-γ reduces the inhibitory effect on HCC cell proliferation and metastasis and the apoptosis-promoting effect on HCC cells and thereby promotes HCC occurrence and development." There is no reference for this sentence. And the grammar needs revision for smooth reading.

Response: We apologize for the grammatical problems with this sentence, and we have revised it according to the reviewer's comments. The sentence "One probable reason is that a low level of IFN-γ reduces the inhibitory effect on HCC cell proliferation and metastasis and the apoptosis-promoting effect on HCC cells and thereby promotes HCC occurrence and development." in the original manuscript has been changed to "One probable reason for this link is that a low level of IFN-γ facilitates HCC cell proliferation and inhibits HCC cell apoptosis, thus promoting HCC occurrence and development" in the revised manuscript.

Moreover, we have added the following two references for this sentence in the revised manuscript.

303 **He YF,** Wang XH, Zhang GM, Chen HT, Zhang H, Feng ZH. Sustained low-level expression of interferon-gamma promotes tumor development: potential insights in tumor prevention and tumor immunotherapy. *Cancer Immunol Immunother* 2005; **54**:891-897 [PMID: 15776283 DOI: 10.1007/s00262-004-0654-1]

304 **Aqbi HF,** Wallace M, Sappal S, Payne KK, Manjili MH. IFN-γ orchestrates tumor elimination, tumor dormancy, tumor escape, and progression. *J Leukoc Biol* 2018; [PMID: 29469956 DOI: 10.1002/JLB.5MIR0917-351R]

15) Page 32: "Another reason is that T helper (Th) cells are important immunomodulatory cells. Th1 and Th2 cells are the main subsets of Th cells. Th1 cells mainly mediate the cellular immune response by secreting cytokines

such as TNF-γ [317]. Th2 cells mainly mediate humoral immunity, which can inhibit the Th1 cellular immune response. Th1 cells are the main immune regulators of tumors. A Thl/Th2 imbalance (increase in Th2 cells and decrease in Thl cells) occurs in the HCC microenvironment, the HCC microenvironment is in a state of immunosuppression [318], and tumor cells undergo immune evasion, which leads to HCC metastasis and recurrence." These sentences are confusing and need revision too.

Response: We apologize for the language problems in the original manuscript. The language presentation has been improved with assistance from American Journal Experts (AJE). The sentences "Another reason is that T helper (Th) cells are important immunomodulatory cells. Th1 and Th2 cells are the main subsets of Th cells. Th1 cells mainly mediate the cellular immune response by secreting cytokines such as TNF-γ [317]. Th2 cells mainly mediate humoral immunity, which can inhibit the Th1 cellular immune response. Th1 cells are the main immune regulators of tumors. A Thl/Th2 imbalance (increase in Th2 cells and decrease in Thl cells) occurs in the HCC microenvironment, the HCC microenvironment is in a state of immunosuppression [318], and tumor cells undergo immune evasion, which leads to HCC metastasis and recurrence." in the original manuscript have been changed to "Another reason is that T helper (Th) cells are vital immunomodulatory cells. Th1 and Th2 cells are the main subsets of Th cells. Th1 cells are the main immune regulators of tumors and primarily mediate the cellular immune response by secreting cytokines such as TNF-γ and they play a role in tumor immune defense [305]. Th2 cells primarily mediate humoral immunity, which can inhibit the Th1-cellular immune response. It has been demonstrated that a Thl/Th2 imbalance (increase in Th2 cells and decrease in Thl cells) occurs in the HCC microenvironment. Thus, the HCC microenvironment is in an immunosuppressive state [306], which enables immune evasion by tumor cells, leading to HCC metastasis and recurrence." in the revised manuscript.

16) Page 33: "to break through the capsule and spread to the outside of the capsule.", is it better for "to break through and eventually spread to the outside of the liver capsule."?

Response: Thank you for this insightful advice. According to this comment, the phrase "to break through the capsule and spread to the outside of the capsule." in the original manuscript has been changed to "to break through and eventually spread to the outside of the liver capsule." in the revised manuscript.

17) Page 33: "The tumor is currently at the expansion stage", "currently" is not suitable here.

Response: Thank you very much for your correction. The sentence "The tumor is currently at the expansion stage," in the original manuscript has been changed to "The tumor is then in the expansion stage," in the revised manuscript.

18) Page 33-34: "Alternatively, most HCC cells synthesize and secrete IL-8, which may affect the biological behavior and metastasis of HCC through autocrine and paracrine signaling.", There is no supporting reference for this sentence.

Response: According to the reviewer's comment, we have added the following reference to this sentence in the revised manuscript.

326 **Harimoto N,** Shirabe K, Abe T, Kajiyama K, Nagaie T, Gion T, Kuroda Y, Maehara Y. Interleukin-8 producing hepatocellular carcinoma with pyrexia. *HPB Surg* 2009; **2009**:461492 [PMID: 19707535 DOI: 10.1155/2009/461492]

Moreover, the sentence "Alternatively, most HCC cells synthesize and secrete IL-8," in the original manuscript has been changed to "Another explanation is that most HCC cells synthesize and secrete IL-8," in the revised manuscript.

19) Page 37-38: “VEGF, as a crucial regulatory factor of angiogenesis, can reflect the level of proliferation, migration and vascular construction of HCC VECs and provides a pathway for HCC cells to metastasize along the blood vessels, which is also an important reason why HCC exhibits high vascular invasion, metastasis tendency and postoperative recurrence [382-384].” These sentences are confusing and need revision.

Response: We are sorry that these sentences were confusing, and we have revised them as recommended. The sentences “VEGF, as a crucial regulatory factor of angiogenesis, can reflect the level of proliferation, migration and vascular construction of HCC VECs and provides a pathway for HCC cells to metastasize along the blood vessels, which is also an important reason why HCC exhibits high vascular invasion, metastasis tendency and postoperative recurrence [382-384].” in the original manuscript have been changed to “VEGF, as a crucial regulatory factor in angiogenesis, plays a regulatory role in the proliferation, migration and vascular construction of HCC VECs. In addition to promoting angiogenesis, VEGF can increase the permeability of the vascular endothelium, making it easier for tumor cells to invade and penetrate blood vessels and consequently facilitating tumor invasion and metastasis. Therefore, the increased expression of VEGF in HCC tissues is an important reason for the high vascular invasion and the high incidence of postoperative recurrence and metastasis of HCC [363-365]” in the revised manuscript.

20) Page 48: “DNA methylation, DNA methylation, histone modifications, genomic imprinting”, DNA methylation repeated, do you mean acetylation?

Response: We apologize for the language problems in the original manuscript. We have deleted the repeated phrase “DNA methylation”. The sentence “The main epigenetic regulatory mechanisms include DNA methylation, DNA methylation, histone modification, genomic imprinting, chromatin remodeling, and noncoding RNA (ncRNA) activity.” in the original

manuscript has been changed to "The main epigenetic regulatory mechanisms include DNA methylation, histone modification, genomic imprinting, chromatin remodeling, and noncoding RNA (ncRNA) activity" in the revised manuscript.

21) Page 50: define "ceRNA"

Response: In accordance with the reviewer's suggestion, the sentence "For example, the oncogenic lncRNA MALAT1 can bind to miR-124 via a ceRNA mechanism and thereby regulate the characteristics of HCC stem cells induced by HBx through the PI3K/Akt pathway [519]. "in the original manuscript has been changed to "For example, the oncogenic lncRNA MALAT1 can bind to miR-124 via a competing endogenous RNA (ceRNA) mechanism, which means that ncRNA with a miRNA response element (MRE) can relieve the miRNA-based inhibition of the target mRNA by competitively combining with the miRNA, and thereby regulate the characteristics of HCC stem cells induced by HBx through the PI3K/Akt pathway [487]." in the revised manuscript.

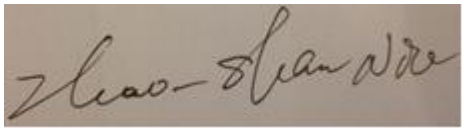
22) Page 51-52: "and HCC invasion and metastasis are complex processes", "and" is not appropriate here, use "but" or "while", etc.

Response: We are very sorry, but we did not find this sentence on Page 51-52.

Thank you for your careful attention to our work. We truly appreciate your efforts in reviewing our manuscript. Your comments have helped us make our review clearer and more comprehensive.

Again, we greatly appreciate your consideration of our paper for publication in the World Journal of Gastroenterology.

Yours sincerely,



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