样式定义：批注文字

Replies to Reviewer's comments:

We appreciate for your kind recommendation and criticism. Specific to your opinion, we have made modification of the manuscript and a point-by-point response, as follows:

In this paper entitled "Exploring the Multifaceted Roles of Lymphatic and Blood Endothelial Cells in the Tumor Microenvironment of Hepatocellular Carcinoma: A Comprehensive Review", Li and colleagues discussed lymphatic endothelial cells (LECs), blood endothelial cells (BECs), angiogenesis, immune modulation, lymphangiogenesis, and metastasis in hepatocellular carcinoma. The manuscript is interesting, but I have several comments that should be addressed before its publication.

1、***In the following paragraph related to the introduction, please add the prevalence of HCC in Asia: "Hepatocellular carcinoma (HCC) is a prevalent form of cancer worldwide, particularly in Asia where the majority of cases are reported."***

Reply: Thank you for your advise. The prevalence of HCC in Asia has been added: "According to the World Cancer Report released in WHO 2020, there were an estimated 905,677 new cases of HCC globally, with 72.5% occurring in Asia alone[1]. Liver cancer accounts for a significant percentage (8.3%) of total cancer-related deaths[2]."

2、***In the following information, the authors should check if the data are correct because the percentage seems very high: "...with 72.5% occurring in Asia alone[1]."***

Reply: Thank for your remind. We have updated and checked the data again. The source is from the website. https://gco.iarc.fr/today/data/factsheets/cancers/11-Liver-fact-sheet.pdf

3、***In the next phrase the reference is missing: "Liver cancer accounts for a significant percentage (8.3%) ……is the primary cause of mortality in solid tumors."***

Reply: We thank the reviewer for pointing this out. The reference has been added in the appropriate position of the manuscript: "Liver cancer accounts for a significant percentage (8.3%) of total cancer-related deaths[2]."

4、***There are several grammatical errors throughout the manuscript, such as tumor immune invasion, intertumoral lymphatic, HGF significantly enhanced the proliferation, among others.***

Reply: We apologize for our grammatical errors, and have made the change as follows: "tumor immune

invasion" has been revised into "tumor invasion"; "intertumoral lymphatic" has been revised into"lymphatic"; "HGF significantly enhanced the proliferation" has been revised into "HGF significantly enhances the proliferation".

5、***With respect to the following phrase, please give some names of tumors, including HCC with its reference, respectively. "While studies have extensively investigated the effects of immune cells like CD cells and regulatory T (Treg) cells on tumors[4-8],"***

Reply: Thank you for your reminding. We have made relevant changes in the revised version. Names of tumors and their references have been given in the appropriate position: While studies have extensively investigated the effects of immune cells on tumors, for example, CD8+ T-cells and NKTcells have been demonstrated to cooperatively promote liver damage and carcinogenesis through interaction with hepatocytes in a NASH-mouse model[3], in human glioblastoma multiforme, macrophage-Associated PGK1 phosphorylation could promotes aerobic glycolysis and tumorigenesis. The CD8+ cytotoxic T cells (CTLs) would kill tumor cells by granule exocytosis and Fas ligand (FasL)-mediated apoptosis induction and induce the cytotoxicity by secreting interferon-γ (IFN-γ) and tumor necrosis factor α (TNFα). Using mouse melanoma models, promoting fatty acids catabolism improves the CD8+ TILs' ability to slow tumor progression. In lung adenocarcinoma, hypoxia upregulates CCL28 to recruit Treg cells which are involved in immune escape of tumor cells. However, Treg cells suppress effector T cells, including CTLs[4-8].

6、***In the introduction section, the authors should add main functions of lymphatic endothelial cells (LECs), blood endothelial cells (BECs) in liver Injury and tumor development.***

Reply: Thank you for your valuable comments. We have added the relevant content in the revised version. Previous studies have demonstrated interactions between LECs, BECs and liver injuries. Chronic inflammation in the liver can induce the proliferation of LECs by inducing the production of chemoattractant cytokines. The increased LECs is proved to be positively correlated with disease severity. Quantity of LECs is increasing during idiopathic portal hypertension, HCV-associated cirrhosis, and primary biliary cirrhosis, LECs changes, seemingly, are reflective of the type of peripheral inflammation[9]. Bacterial products such as LPS, which is increased in cirrhosis, activate Nf-Kb in LECs and thus consequently upregulate Prox1 and VEGFR-3. TGF-β1 is released in the TME of HCC to increase the expression of CD105 in BECs, enhancing the invasion and metastasis of liver cancer cells by inducing neoangiogenesis.

7、***I recommend the next good reference (PMID: 36612019) for the introduction section of this interesting manuscript.***

Reply: Thank you for recommending this excellent manuscript to us, and having cited this reference

into the introduction.

8、***In figure 1, the effects of growth factors on lymphangiogenesis should be briefly described in figure legend.***

Reply: Thank you for your suggestion. A brief description has added in the figure legend as follows: VEGF-C, FGFs, HGF, ANGPTs, AM promote the survival, growth, and migratory ability of LECs; NRP2 forms a complex with VEGFR-3 upon binding with VEGF-C/D, enhancing lymphangiogenesis; S1P induces migration, sprouting, capillary-like tube formation of LECs; HGF, PDGF are directly involved in lymphangiogenesis. HGF indirectly promotes VEGF-C/D expression, contributing to lymphangiogenesis.

9、***It is important to describe the effects and the processes, focusing on HCC or give names of cancers according to information written in the manuscript, because it is confusing and could be considered as information related to HCC. For example, in the next phrases, indicate the type of cancer: "LECs, the process can be inhibited by c-Met, the receptor of HGF[26]." "Overexpression of ANGPTs promotes lymphangiogenesis in adult tissue in vivo, as observed in experimental tumor models[28,29]."***

Reply: Thank you for your proposal, and the specific information has been added in the revised manuscript. What's more, in oral squamous cell carcinoma, HGF significantly enhances the proliferation, migration, invasion and tube formation of LECs, the process can be inhibited by downregulating the expression of c-Met, the receptor of HGF[10]. Overexpression of ANGPTs promotes lymphangiogenesis in adult tissue in vivo, as observed in experimental pancreatic cancer models[11, 12].

10、***The following information should be reviewed in order to confirm if it is correct according to reference 36: "Folkman[36] proposed the hypothesis that angiogenesis is essential for the development and growth of solid tumors beyond a size of 1-2mm3."***

Reply: We apologize for our error. The following information has been revised: In 1971, Folkman[13] proposed the hypothesis that angiogenesis is essential for the development and growth of solid tumors beyond a size of 2-3 mm^3.

11、***In the legend of table 1, define all markers and clarify in the manuscript if these markers were reported in HCC or indicate in which type of cancers are expressed.***

Reply: The definition of the markers and types of cancers have been added in the table 1.

Table 1 Common Markers of Lymphatic endothelial cells and Blood endothelial cells

Marker	Definition	Expressed on Cells	Addition	Types of cancer	Ref.
PROX-1	an evolutionarily conserved class of atypical homeodomain proteins	LECs	Located in the nucleus and promoting lymphangiogenesis	Breast cancer、Melanoma、Glioblastoma	[50-56]
PDPN	a transmembrane mucin type O-glycoprotein	LECs	Functioning in the downstream of PROX-1	Angiosarcomas、Melanoma、Colorectal carcinoma、Breast cancer、Osteosarcoma	[57-60]
LYVE-1	one of the hyaluronan-binding glyco-protein receptors	LECs	Acting with VEGFR and PDGFR in the LECs	Oral oncogenesis、Lung cancer	[62-66]
VEGFR-3	An receptor tyrosine kinase	LECs / BECs	Functioning by activating RAS/RAF-1/MEK/ERK signaling pathway	Gastric cancer、Intrahepatic cholangiocarcinoma、Colorectal carcinoma	[67,68]
CD31	One of the immunoglobulin superfamily	LECs / BECs	Promoting tumor angiogenesis by regulating TME indirectly	Breast cancer、Melanoma、Gastric cancer	[71, 73-75]
CD105	Homodimeric transmembrane glycoprotein, a coreceptor for ligands of the TGF-β family	BECs	Also called Endoglin	Esophageal squamous cell carcinoma、Colorectal carcinoma	[76-79]

12、***According to the next information in the phrase, the reference 44 is not related to it, please add the appropriate one: "PROX-1, a transcription factor, is regarded as a constitutive marker of LECs due to its pivotal role in lymphangiogenesis[44-47]." The same situation for the reference 39, which does not mention PROX-1: "It is consistently located in the nuclei of all LECs, regardless of their physiological or pathological state[39]."***

Reply: We apologize for our neglect. The references have altered: PROX-1, a transcription factor, is regarded as a constitutive marker of LECs due to its pivotal role in lymphangiogenesis[14, 15]. It is consistently located in the nuclei of all LECs, regardless of their physiological or pathological state[16].

13、***The reference 60 is not related to the next phrase, please add the appropriate one: "When VEGF-C/D binds …….. RAS-RAF-1-MEK-ERK signaling pathway and ultimately promoting lymphangiogenesis[60]."***

Reply: We apologize for our cursoriness. The reference has been modified: When VEGF-C/D binds to VEGFR-3, it triggers dimerization and transphosphorylation of the receptor, leading to activation of the RAS-RAF-1-MEK-ERK signaling pathway and ultimately promoting lymphangiogenesis[17, 18].

14、***The author's name in the following sentence is wrong: "Recently, Michael et al.[63] investigated EVT801, a novel selective VEGFR-3 inhibitor that specifically targets….."***

Reply: We apologize for our mistake. The author's name has been modified: Paillasse et al[18],investigated EVT801, a novel selective VEGFR-3 inhibitor that specifically targets VEGFR-3-positive tumors and tumors with a VEGFR-3-positive TME.

15、***Figure 2 should contain a brief description in the figure legend. -Please add the references for the following phrases: " Tie is the receptor for Ang, containing Tie1…….expressed only in BECs and acts on BECs in an autocrine form, is a partial competitive antagonist of Ang1 against Tie2."***

Reply: Thank you for your previous advice. The description of Figure 2 has been added as follows: Proinflammatory mediators increase the production of CCL1 by LECs and enhance the migration of tumor cells towards LECs. CCL1/CCR8 Axis is involved in recruiting Treg cells into the TME and converting CD4+ T cells into Treg cells. CXCR4 expression caused by products of the microbiota and chronic hypoxia stimulates tumor proliferation and migration of LECs into lymphatic vessels. VEGF can enhances the effect of CXCL12/CXCR4 Axis. The CCL21/CCR7 axis induces EMT and promotes proliferation of tumor cells, LECs and ECM.

16、***There is scarce information in the heading "Role of LECs and BECs in tumor metastasis" related to the role of BECs and BECs in tumor metastasis, especially related to HCC, please add more information.***

Reply: More information has been added. Besides direct association with cancer development, BECs are one of the sources of cancer-associated fibroblasts (CAFs)[19]. The heterogeneous group of CAFs is the main inductor of migration and invasion abilities of cancer cells. Nutrition and oxygen are provided to HCC contributing to its growth and development by discontinuous BECs. BECs are also involved in intravasation, which allows HCC cells to translocate into the blood vessel lumen[20].

17、***The next heading is incomplete "SPECIAL FOCUS ON HCC" Is it related to LECs and BECs?***

Reply: The heading has been supplied:"**SPECIAL FOCUS ON LECS AND BECS IN THE TME OF HCC"**. The paragraph is related to LECs and BECs. The manuscript describes that LECs in the TME of HCC take advantages in participating in the nearby arising immune responses, chronic inflammation induces the production of chemoattractant cytokines and activate NF-κB in LECs, influencing lymphangiogenesis, HCC-released TGF-β1 promotes the expression of CD105 in BECs, acting as a promoter of tumor angiogenesis and so on.

18、***The references 44, 92 and 93 are not related to the liver, please add the appropriate ones: "Preliminary results have been found in liver cancer about the correlation between lymphangiogenesis and cancer prognosis[44,92,93]."***

Reply: We have fixed the error. The unrelated references have been deleted.

19、***In the manuscript there is little information about immune modulation in HCC. Please add more information. In addition, it should include more information regarding the TME components in HCC, for example factors secreted and effects.***

Reply: More information has been added in "**INTRODUCTION**": Mesenchymal stem cells (MSCs) secrete hepatocyte growth factor (HGF), IDO, NO, PGE2 and TGFβ, which inhibit cytotoxic activity and differentiation of T helper 1 (TH1) cells. IL-10 and PGE2 secreted by MSC in the TME impair dendritic cell (DC) maturation, which contributes to less efficient T cell activation[21]. In the case of HCC, the action of IL-4, IL-13, IL-10 and activation of the Tolllike receptors diminish antigenpresenting activity[22]. TGFβ and thymic stromal lymphopoietin (TSLP), which inhibit T cells and promote T cell skewing towards a TH2 phenotype, respectively. CAFs also secrete inflammatory cytokines, including CXC-chemokine ligand 8 (CXCL8), IL-4 and IL-6, which further suppress T cell activity. It's worth to mention that several chemokines secreted by cancer-associated fibroblasts (CAFs) in TME inhibited immune cells: CXCL12 repels T cells, CXCL13 recruits B cells, and CCL2, CCL3,

CCL4 and CCL5 recruit myeloid cells, including macrophages and myeloid-derived suppressor cells (MDSCs), ECM, extracellular matrix[23]. ECM secreted by stromal cells in TME also significantly influences antitumour immune responses.

20、***In the abstract section, the authors propose to shed light on future research directions and potential therapeutic targets for HCC treatment. However, there is scarce information, therefore it should be added.***

Reply: Relevant information has been added in the proper place. "Mechanisms of BECs inhibiting antitumor immunity is still in uncertainty so far. Considering that blocking the process of BECs inhibiting antitumor immunity can avoid tumors development via regulating self-immunity without those intolerable complications, it is worth to research the explicit mechanisms. How it specificly works needs more research to testify. What's more, considering that different high risk factors causing HCC such as chronicly infecting Hepatitis B Virus or Nonalcoholic steatohepatitis have different mechanisms, whether the function the ingredients of TME takes is distinct during the development of HCC, which needs further research..While we now know that BECs and LECs can play a much more complex role than offering nutrition and forming the conduits of tumor cell metastasis more research is needed to decipher the mechanisms involved, and effective therapies targeting BECs and LECs may be developed in future."

Thanks again for your professional review and criticism. Hope for your further recommendation.

References

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