RESPONSE TO REVIEWER:

Thank you for your constructive comments on our manuscript. The reviewer's comments were very helpful. Our responses to the reviewer's suggestions have been provided below. We wish to express our appreciation to the Reviewers for their insightful comments, which have helped us significantly improve our manuscript.

Reviewer's code: 02992981

Comment 1 :

It is strongly suggested that using a figure or table indicates the differences in biological characteristics including morphology, origin, biological markers, function among fibroblast, cancer-associated fibroblast, myofibroblast, HSCs and quiescent HSCs for easy to read.

Response to the comment: We appreciate the reviewer's suggestion.

In accordance with the Reviewer's comment, we have attached table 1 and revised the text in INTRODUCTION section (p. 5, lines 23) as:

Therefore, NTF, which are activated HSCs from cirrhotic tissue, may have already undergone a genetic change that affects surface markers. Collagen 11A1 expression is a remarkable biomarker of human carcinoma-associated stromal cells [13]. It was reported that even without exposure to cancer cells, the tumor promoting characteristics of CAFs can be stably maintained [14].

We revised some of the text in the INTRODUCTION on p. 6, lines 7 as:

Furthermore, activated HSCs can be stimulated by cancer cells, which then become CAFs. Details of differentiation into quiescent HSCs, HSCs, and CAFs is shown in the Table 1. Quiescent HSCs were characterized by the stored vitamin A with fat droplets [16] and are derived from the mesoderm [17]. HSCs have a function in wound healing and fibroblast production. Characterization of CAFs revealed that they were affected by cancer cells through "crosstalk".

Comment 2:

This review mainly focus on the roles of cancer-associated fibroblast in HCC progression, but little information on other constitutes of tumor microenvironment like HSCs, invading inflammatory/immune cells, macrophage, endothelial cells, extracellular matrix proteins, proteolytic enzymes, hypoxia, matrix stiffness etc. So it is suggested to modify the title of this article to match the presented contents.

Response to the comment: We appreciate the reviewer's suggestion

The final title has been modified to match the presented content.
We change the title from "**Role of tumor microenvironment and cancer-associated fibroblasts in hepatocellular carcinoma progression"** to "**Role of cancer-associated fibroblasts in hepatocellular carcinoma**"

Comment 3:

It is priority for reviewing advance of CAF in HCC to cite more published references in last three years. But most references in this review are old, and require to be updated.

Response to the comment: We appreciate the reviewer's suggestion

The reference list has been updated per your suggestion.
We have included eleven additional references that are listed below.

8 Song J, Ge Z, Yang X, Luo Q, Wang C, You H, Ge T, Deng Y, Lin H, Cui Y, Chu W, Yao M, Zhang Z, Gu J, Fan J, Qin W. Hepatic stellate cells activated by acidic tumor microenvironment promote the metastasis of hepatocellular carcinoma via osteopontin. *Cancer Lett* 2015; **356**(2 Pt B): 713-720 [PMID: 25449435 DOI: 10.1016/j.canlet.2014.10.021]

12 Martin M, Ancey PB, Cros MP, Durand G, Le Calvez-Kelm F, Hernandez-Vargas H, Herceg Z. Dynamic imbalance between cancer cell subpopulations induced by transforming growth factor beta (TGF-beta) is associated with a DNA methylome switch. *BMC Genomics* 2014; **15**: 435 [PMID: 24898317 PMCID: PMC4070873 DOI: 10.1186/1471-2164-15-435]

13 Vazquez-Villa F, Garcia-Ocana M, Galvan JA, Garcia-Martinez J, Garcia-Pravia C, Menendez-Rodriguez P, Gonzalez-del Rey C, Barneo-Serra L, de Los Toyos JR. COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression. *Tumour Biol* 2015; **36**(4): 2213-2222 [PMID: 25761876 DOI: 10.1007/s13277-015-3295-4]

31 Wallace MC, Friedman SL. Hepatic fibrosis and the microenvironment: fertile soil for hepatocellular carcinoma development. *Gene Expr* 2014; **16**(2): 77-84 [PMID: 24801168 DOI: 10.3727/105221614X13919976902057]

39 Kim GJ, Rhee H, Yoo JE, Ko JE, Lee JS, Kim H, Choi JS, Park YN. Increased expression of CCN2, epithelial

membrane antigen, and fibroblast activation protein in hepatocellular carcinoma with fibrous stroma showing aggressive behavior. *PLoS One* 2014; **9**(8): e105094 [PMID: 25126747 PMCID: PMC4134271 DOI: 10.1371/journal.pone.0105094]

40 Eiro N, Vizoso FJ. Importance of tumor/stroma interactions in prognosis of hepatocellular carcinoma. *Hepatobiliary Surg Nutr* 2014; **3**(2): 98-101 [PMID: 24812604 PMCID: PMC3999424 DOI: 10.3978/j.issn.2304-3881.2014.02.12]

71 Ding S, Chen G, Zhang W, Xing C, Xu X, Xie H, Lu A, Chen K, Guo H, Ren Z, Zheng S, Zhou L. MRC-5 fibroblast-conditioned medium influences multiple pathways regulating invasion, migration, proliferation, and apoptosis in hepatocellular carcinoma. *J Transl Med* 2015; **13**: 237 [PMID: 26198300 PMCID: PMC4508812 DOI: 10.1186/s12967-015-0588-8]

77 Albrengues J, Bourget I, Pons C, Butet V, Hofman P, Tartare-Deckert S, Feral CC, Meneguzzi G, Gaggioli C. LIF mediates proinvasive activation of stromal fibroblasts in cancer. *Cell Rep* 2014; **7**(5): 1664-1678 [PMID: 24857661 DOI: 10.1016/j.celrep.2014.04.036]

78 Satoyoshi R, Kuriyama S, Aiba N, Yashiro M, Tanaka M. Asporin activates coordinated invasion of scirrhous gastric cancer and cancer-associated fibroblasts. *Oncogene* 2015; **34**(5): 650-660 [PMID: 24441039 DOI: 10.1038/onc.2013.584]

79 Albrengues J, Bertero T, Grasset E, Bonan S, Maiel M, Bourget I, Philippe C, Herraiz Serrano C, Benamar S, Croce O, Sanz-Moreno V, Meneguzzi G, Feral CC, Cristofari G, Gaggioli C. Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts. *Nat Commun* 2015; **6**: 10204 [PMID: 26667266 PMCID: PMC4682161 DOI: 10.1038/ncomms10204]

81 Procopio MG, Laszlo C, Al Labban D, Kim DE, Bordignon P, Jo SH, Goruppi S, Menietti E, Ostano P, Ala U, Provero P, Hoetzenecker W, Neel V, Kilarski WW, Swartz MA, Brisken C, Lefort K, Dotto GP. Combined CSL and p53 downregulation promotes cancer-associated fibroblast activation. *Nat Cell Biol* 2015; **17**(9): 1193-1204 [PMID: 26302407 PMCID: PMC4699446 DOI: 10.1038/ncb3228]

82 Procopio MG, Laszlo C, Dotto GP. CSL-p53: From senescence to CAF activation. *Cell Cycle* 2016; **15**(4): 485-486 [PMID: 26735629 DOI: 10.1080/15384101.2015.1130091]

83 Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat Med* 2015; **21**(12): 1424-1435 [PMID: 26646499 PMCID: PMC4748967 DOI: 10.1038/nm.4000]

84 Sprinzl MF, Galle PR. Immune control in hepatocellular carcinoma development and progression: role of stromal cells. *Semin Liver Dis* 2014; **34**(4): 376-388 [PMID: 25369300 DOI: 10.1055/s-0034-1394138]

127 Riehle KJ, Yeh MM, Yu JJ, Kenerson HL, Harris WP, Park JO, Yeung RS. mTORC1 and FGFR1 signaling in fibrolamellar hepatocellular carcinoma. *Mod Pathol* 2015; **28**(1): 103-110 [PMID: 24925055 DOI: 10.1038/modpathol.2014.78]

In accordance with the Reviewer's comment, we have revised the text in the Crosstalk section on p. 8, line 1 as:

“CAFs stimulate malignant cell proliferation by providing different types of growth factors and

cytokines in a context-dependent manner[20] such as SDF-1[42-45], HGF[46-48], members of the epidermal growth factor family[49], fibroblast growth factor (FGF)[50, 51], Wnt families[52], forkhead box F1[53], IL-6[54-56], TGF-β[57, 58], and EGF. When HCC cells are co-cultured with CAFs, CAFs induced by TIMP-1 repress HCC apoptosis with an increased Bcl-2/BAX ratio through SDF-1/CXCR4/PI3K/AKT signaling[44]. Moreover, CAFs upregulated gene expressions of TGF-β and FAP, whereas NTFs did not induce the expression of either gene [4].”

We have revised some text in the Crosstalk section on p. 8, line 21 as

Crosstalk between TGF-β and PDGF signaling supports epithelial mesenchymal transition (EMT), which is crucial for tumor growth and the acquisition of an invasive phenotype [70]. MRC-5 fibroblast-conditioned medium influences multiple pathways regulating invasion in HCC [71].

We have revised some text in the Crosstalk section on p. 9, line 10 as:

“These functions of CAFs in supporting HCC growth were confirmed by *in vitro* experiments involving co-culture of HCC cell lines with CAFs [4]. Remarkably, the activation of CAFs was maintained after their isolation from cells of various cancer types such as squamous skin carcinoma, lung carcinoma, breast carcinoma, and scirrhous gastric cancer [76-78]. Exposure to leukemia inhibitory factor initiates an epigenetic switch causing the constitutive activation of JAK1/STAT3 signaling, which results in sustained activation of CAFs[79]. DNA methylation plays critical roles in the control of sustained and constitutive activation of signaling pathways[80]. CAF activation is accompanied by stromal cell senescence [81, 82]. Concomitant loss of CSL (also known as RBP-Jk) and p53 overcomes fibroblast senescence, enhances expression of CAF effectors, and promotes stromal and cancer cell expansion [81] through β-galactosidase [83], IL-6, and IL-8 [82] respectively. Xenografts in nude mice also demonstrated *in vivo* tumor growth enhancement by CAFs [48].”

Reviewer's code: 02992981

Comment :

Interesting review. May also benefit from a paragraph discussing the fibrolamellar variant of hepatocellular carcinoma

Response to the comment: We appreciate the reviewer's suggestion

We have revised some text in the CAFs as therapeutic target section on p. 12, line 18 as

Fibrolamellar hepatocellular carcinoma was surrounded by laminated fibrous stroma[126]. It was reported the overexpression of fibroblast growth factor receptor 1 in fibrolamellar hepatocellular carcinoma[127]. CAFs stimulate tumor cells by FGF[50, 51] and produced fibrosis. Treatment targeting CAFs is might be effective in a fibrolamellar hepatocellular carcinoma.

Thank you once again for your comments and suggestions. We believe that the revised manuscript is suitable for publication.