OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

The Role Of Hepatic Stellate Cells In Promoting Liver Metastasis Of Colorectal Carcinoma

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Wen-Hai Huang[1,*]
Min-Wei Zhou[1,*]
Yan-Feng Zhu[2,*]
Jian-Bin Xiang[1]
Zhen-Yang Li[1]
Zi-Hao Wang[1]
Yi-Ming Zhou[1]
Yi Yang[1]
Zong-You Chen[1]
Xiao-Dong Gu[1]

[1]Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, People's Republic of China; [2]Department of Nursing, Huashan Hospital, Fudan University, Shanghai 200040, People's Republic of China

*These authors contributed equally to this work

Purpose: Colorectal cancer (CRC) is the most common malignancy in the gastrointestinal tract. The liver is the most common location of CRC metastases, which are the main causes of CRC-related death. However, the mechanisms underlying metastasis of CRC to the liver have not been characterized, resulting in therapeutic challenges.

Methods: The effects of hepatic stellate cells (HSCs) on T cells were evaluated using in vitro mixed lymphocyte reactions (MLRs) and cytokine production assays. HSC-induced CT26 cell migration and proliferation were evaluated in vitro and in vivo.

Results: HSCs induced T cell hypo-responsiveness, promoted T cell apoptosis, and induced regulatory T cell expansion in vitro. IL-2 and IL-4 were significantly lower in MLRs incubated with HSCs. Supernatants of MLRs with HSCs promoted CT26 cell proliferation and migration. Furthermore, the presence of HSCs increased the number of liver metastases and promoted proliferation of liver metastatic tumor cells in vivo.

Conclusion: HSCs may contribute to an immunosuppressive liver microenvironment, resulting in a favorable environment for the colonization of CRC cells in the liver. These findings highlight a potential strategy for treatment of CRC liver metastases.

Keywords: colorectal cancer, liver metastasis, hepatic stellate cells, dendritic cells, T cells

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and has increased in prevalence in recent years.[1] CRC frequently metastasizes to the liver, and liver resection and perioperative chemotherapy are the primary means of therapeutic intervention for these tumors. The median survival time for patients with untreated CRC and liver metastases is 6.9 months, and 5-year survival rates following liver resection range from 30% to 50%. Several recent studies have aimed to evaluate the mechanisms responsible for liver metastasis. However, the mechanisms underlying liver metastasis of CRC have not been characterized, resulting in challenges to development of effective therapies.[2–4]

In 1889, Paget proposed the "seed and soil" theory of metastatic dissemination. Paget suggested that the site of metastasis depended on the affinity of the tumor for the microenvironment.[5] To evaluate the hepatic microenvironment, we previously analyzed liver non-parenchymal cells in mice and showed that hepatic stellate cells (HSCs), which store retinol and participate in repair and fibrogenesis during liver injury, play a role in immune regulation.[6] Recent studies of HSCs have focused on liver injury, liver fibrosis, and liver regeneration. Several studies have shown that HSCs exhibit immunomodulatory activity and can prolong allograft survival.[7,8]

Correspondence: Zong-You Chen; Xiao-Dong Gu
Department of General Surgery, Huashan Hospital, Fudan University, 12 Wulumuqi Middle Road, Shanghai 200040, People's Republic of China
Tel +86 21 52887330; +86 21 52887333
Fax +86 21 62495490
Email zongyouc@sohu.com; gxdgxd737@163.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/OTT.S214409

Furthermore, HSCs have been shown to promote onset and progression of hepatocellular carcinomas.[9,10] We previously showed that quiescent HSCs express low levels of immune surface molecules. Priming HSCs with IFN-γ resulted in marked upregulation of the inhibitory co-stimulatory molecule B7-H1, potentially through activation of the MEK/ERK pathway.[6] However, the mechanisms by which HSCs promote metastasis of CRC cells to the liver have not been elucidated.

In this study, we demonstrate that HSCs induce T cell hypo-responsiveness and expand regulatory T (Treg) cells. Moreover, HSCs were shown to play an immunosuppressant role in the hepatic microenvironment and promote CRC metastasis to the liver.

Materials And Methods

Animals

BALB/c mice were obtained from the Shanghai SLAC Laboratory Animal Company. All mice were maintained in a specific pathogen-free environment at Huashan Hospital. Animals were fed standard chow ad libitum and subjected to experiments at 7–9 weeks of age. The animal study protocol was approved by the ethics committee of Huashan Hospital. All experiments were performed following the Huashan Hospital Laboratory Animal Centre care guidelines.

Isolation, Culture, And Identification Of HSCs

HSCs were isolated from murine livers as previously described.[11] Briefly, the livers were perfused through the portal vein with collagenase IV (Life Technologies, Grand Island, NY, USA). The smashed cells were filtered through a nylon mesh. HSCs were purified by Percoll density gradient centrifugation (Sigma-Aldrich, St. Louis, MO, USA) and cultured in complete medium supplemented with 20% FBS (Gibco, Gaithersburg, MD, USA) for 7 to 14 days, unless otherwise indicated. The purity of HSCs ranged from 90% to 95%, as measured by desmin immunostaining and typical appearance of lipid droplets under a light microscope.

Isolation And Culture Of Dendritic Cells (DCs)

DCs were generated from bone marrow progenitor cells as previously described.[12] Bone marrow cells were extracted from femurs and tibias of BALB/c mice, and erythrocytes were lysed using ammonium chloride. The cells were cultured in 24-well plates (1×10^6 cells/well) in 1 mL of RPMI 1640 (Gibco) supplemented with 10% FBS and 10 ng/mL recombinant granulocyte-macrophage colony stimulating factor (R&D Systems, Minneapolis, MN, USA). All cultures were incubated at 37°C in 5% humidified CO_2. Nonadherent granulocytes were removed after 48 hrs of culture. Half of the media was exchanged every 48 hrs. After 6 days of culture, 1 μg/mL lipopolysaccharide (Sigma-Aldrich) was added to the culture media for 18 hrs to allow for maturation. The purity of DC preparations was routinely monitored by flow cytometry using an anti-CD11c monoclonal antibody (mAb) (eBioscience, San Diego, CA, USA). CD11c+ cells were enriched to >85%.

Tumor Antigen Uptake

The mouse colon carcinoma CT26 cell line was purchased from American Type Culture Collection and cultured in DMEM (Gibco) supplemented with 10% FBS. On day 6 of DC culture, CT26 mouse colon cancer cell lysates were added to the culture medium cultures consisted of a DC-to-CT26 ratio of 1:10 for 18 hrs at 37°C in 5% humidified CO_2. CT26 mouse colon cancer cell lysates were obtained through six freeze/thaw cycles in PBS (Sigma-Aldrich).

Mixed Lymphocyte Reactions (MLRs)

For primary MLRs, nylon wool-eluted spleen T cells (2×10^5) from BALB/c mice were used as responders, and γ-irradiated (20 Gy) tumor antigen-pulsed DCs derived from BALB/c bone marrow were used as stimulators. Cultures were maintained in complete medium for 3 days at 37°C in 5% humidified CO_2. [^{3}H]-TdR (0.5μCi/well) was added for the final 18 hrs of culture. Cells were harvested onto glass fiber disks using an automated system, and incorporation of [^{3}H]-TdR into DNA was assessed using a Wallac 1450 liquid scintillation counter (PerkinElmer, Boston, MA, USA). Results are expressed as mean counts per minute (cpm)±SD. To examine the effect of HSCs on T cell proliferation, γ-irradiated (50 Gy) HSCs were added at the beginning of culture. T cells were cultured with tumor antigen-pulsed DCs at a ratio of 10:1 for 3 days. HSCs were added into the culture at a HSCs: T cells ratio of 1:20, 1:40, 1:80, or 1:160.

Flow Cytometric Analysis

Expression of cell surface molecules was detected using a FACScan (BD Biosciences, San Jose, CA, USA), and analyzed using CellQuest software (BD Biosciences). Cells were stained with the following monoclonal antibodies:

FITC-CD25 (eBioscience), PE-Cy5-CD4, and FITC-CD3 (BD Biosciences). Isotype-matched irrelevant mAbs were used as negative controls. Apoptosis was assessed using PE-Annexin V staining (BD Biosciences). Foxp3 staining was performed using fixation and permeabilization buffers contained in the Foxp3 kit according to manufacturer's instructions (eBioscience).

CCK8 Assay

CT26 cells were plated in triplicate at 4×10^3 cells/well in 96-well plates. MLR supernatants were collected and added to the wells. At 24, 48, 72, and 96 hrs, 10 μL of CCK8 solution (Dojindo, Kumamoto, Japan) was added to each well. Absorbance was detected at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) following incubation at 37°C for 2 hrs.

Transwell Migration Assay

Transwell migration chambers (Corning Inc., Corning, NY, USA) were used to evaluate the migration of CT26 cells. CT26 cells (5×10^4) in 200 μL of serum-free medium were added to the upper chamber, and supernatants (800 μL) collected from MLR experiments with or without HSCs were added to the lower chamber. After incubation for 24 hrs, cells on the upper surface of the membrane were removed using a cotton swab. The remaining cells were fixed in methanol, stained using crystal violet, and air-dried. The number of migrating cells on each membrane was counted in five random fields per well using a light microscope.

Cytokine Analysis

IL-2, IL-4, IL-10, and IFN-γ levels in MLR culture supernatants were measured using ELISA kits according to the manufacturer's instructions (Jingmei Biotech Limited Company, Shenzhen, People's Republic of China). The minimum detectable dose of IL-2 is 15.6 pg/mL. The minimum detectable dose of IL-4 is 7.8 pg/mL. The minimum detectable dose of IL-10 is 15.6 pg/mL. The minimum detectable dose of IFN-γ is 9.4 pg/mL. MLR culture supernatants were harvested at the end of the third day of culture.

Animal Model Of CRC Liver Metastasis

A murine model of CRC-derived liver metastasis was established as previously described.[13] CT26 cells were washed twice with 0.5 M PBS after trypsinization and suspended in PBS. The animal model was established under anesthesia using isoflurane (Abbott Laboratories, Abbott Park, IL, USA). For the laparotomy, a median incision was made and a 30-gauge needle was used to puncture the portal vein. A 0.1 mL cell suspension containing 1×10^6 CT26 cells, or a mixture of 1×10^6 CT 26 cells and 5×10^5 HSCs, was injected to achieve a liver metastasis model.

Immunohistochemistry

Immunohistochemistry was performed in liver metastatic tumor samples from murine model. Tissue paraffin sections were deparaffinized, rehydrated and pre-treated with 10 mM sodium citrate buffer at a sub-boiling temperature for 10 mins to unmask the antigen. The sections were subsequently incubated with 3% H_2O_2 for 10 mins at room temperature and dark conditions to block endogenous peroxidase activity, followed by incubation with blocking solution for 1 hr to avoid unspecific binding of the primary antibody. The sections were then incubated overnight at 4ºC with the anti-PCNA antibody (Cell Signaling Technology, Danvers, MA, USA) followed by incubation for 30 mins at room temperature with a biotinylated ECL anti-rabbit IgG (GE Healthcare, Chalfont St. Giles, UK). The color was developed using the diaminobenzidine substrate (Roche Diagnostics, Mannheim, Germany), and the sections were counterstained with hematoxylin. Slides were visualized and photographed using a Leica DM2500 light microscope (Leica Microsystems Inc., Buffalo Grove, IL, USA).

Statistical Analysis

All data analyses were conducted using SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). Comparative analysis of the data was performed using one-way ANOVA or Student's *t*-test. Differences were considered statistically significant when $P < 0.05$.

Results

DCs Effectively Took Up Tumor Antigens

To examine the effects of tumor antigen uptake by DCs, DCs were pulsed with tumor antigens as stimulators in MLRs. On day 6 of DC culture, tumor antigens were added to the culture at a DC:CT26 ratio of 1:10 for 18 hrs at 37°C in 5% humidified CO_2. The results showed that DCs that did not take up tumor antigens did not promote T cell proliferation. ($P<0.05$) (Figure 1).

HSCs Inhibited T cell Proliferation

To evaluate the effects of HSCs on T cell proliferation, HSCs were added to an MLR culture containing splenic T cells stimulated by DCs pulsed with tumor antigens. HSCs

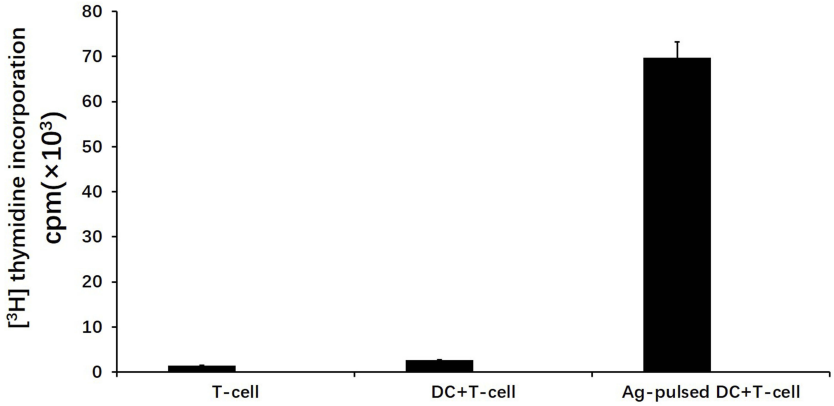

Figure 1 DCs effectively took up tumor antigens. DCs that did not take up tumor antigens did not promote T cell proliferation. DCs able to effectively take up tumor antigens promoted T cell proliferation to a significantly greater extent than DCs that did not take up tumor antigens (P<0.05). The ratio of T cells to DCs was 20:1.

inhibited T cell proliferation (P<0.05) (Figure 2A) in an HSC:T cell ratio-dependent manner (Figure 2B).

HSCs Induced T Cell Apoptosis

We hypothesized that HSC-mediated inhibition of T cell proliferation may have resulted from apoptosis of activated T cells. To test this hypothesis, splenic T cells were cultured for 3 days with tumor antigen-pulsed DCs in the presence or absence of HSCs. The cells were double-stained with anti-Annexin V mAb and anti-CD3 mAb. As shown in Figure 3, the proportion of cells that were double-positive for Annexin V and CD3 staining was higher in the group cultured with HSCs than that in the group cultured without HSCs (P<0.05). These results confirmed that HSCs increased T cell apoptosis.

HSCs Promoted Treg Cell Expansion

To examine the effects of HSCs on Treg cell activity, Treg cells were quantified in MLRs in which splenic T cells were cultured for 3 days with tumor antigen-pulsed DCs in the presence of HSCs. The percentage of CD4+CD25+ FoxP3+ cells in MLR cultures with HSCs was higher than that in MLR cultures without HSCs (P<0.05) (Figure 4B). This result confirmed that HSCs promoted Treg cell expansion.

Cytokine Levels In Supernatants Of MLRs Were Altered By HSCs

To evaluate the effects of HSCs on cytokine secretion, IL-2, IL-4, IL-10, and IFN-γ levels were quantified in MLR culture supernatants using ELISA. The results showed that the expression levels of IL-2 and IL-4 in supernatants of MLRs with HSCs were significantly lower than those in supernatants of MLRs without HSCs (P<0.05) (Figure 5). No differences were observed for IL-10 or IFN-γ expression in MLRs with or without HSCs.

MLR Supernatants Promoted CT26 Cell Proliferation And Migration

To determine the effects of MLR supernatants on CT26 cells, CCK8 assay was used to evaluate CT26 cell

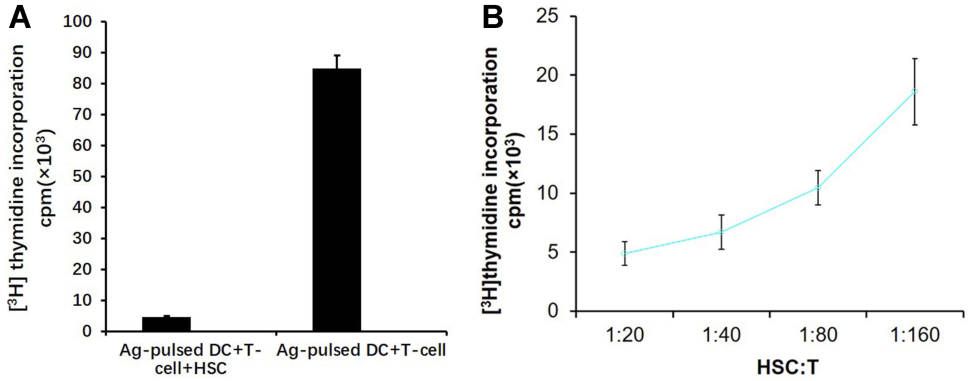

Figure 2 (**A**) HSCs inhibited T lcell proliferation. The ratio of T cells to DCs to HSCs was 20:2:1. (**B**) In each group, 2×10^5 nylon wool-eluted spleen T cells from BALB/c mice were cultured with DCs in the presence or absence of HSCs. The ratio of T cells to DCs (10:1) was the same for all conditions, but the ratio of HSCs to T cells varied. HSCs inhibited T cell proliferation in a dose-dependent manner.

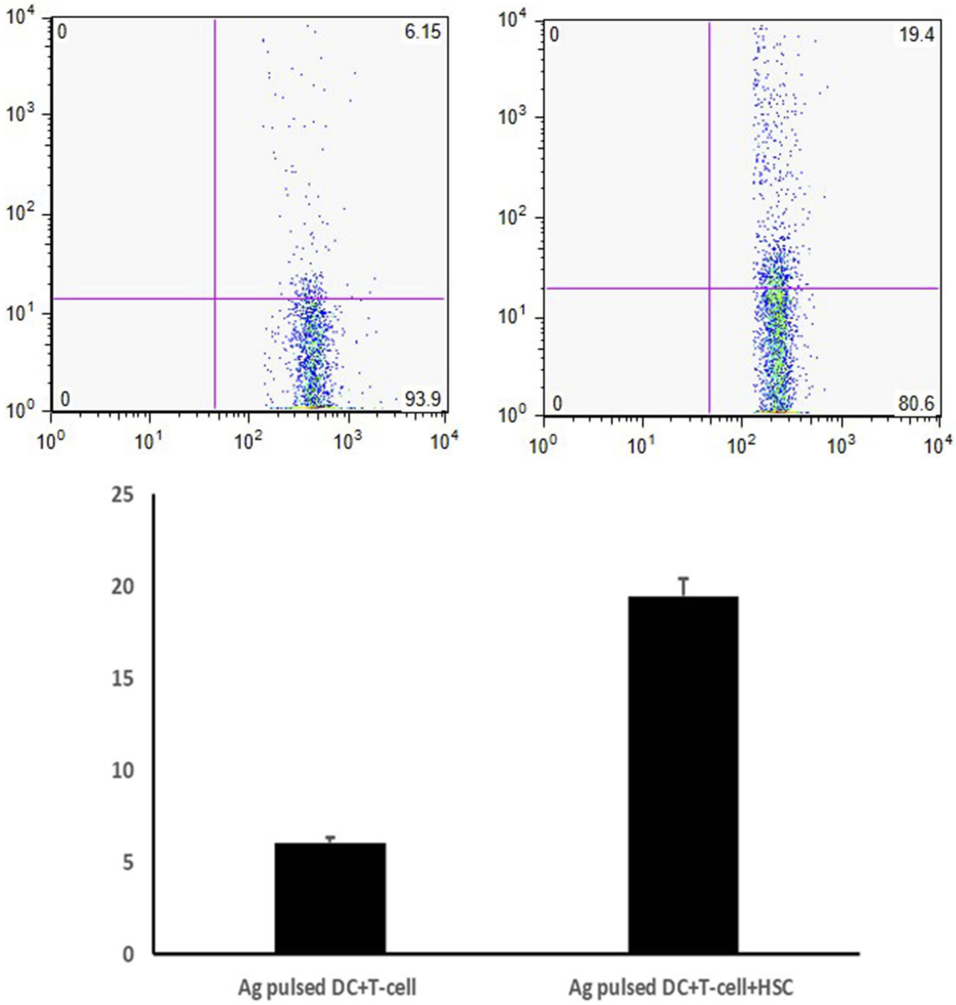

Figure 3 Nylon wool-eluted spleen T cells from BALB/c mice were cultured with DCs in the presence or absence of HSCs. The ratio of T cells to DCs to HSCs was 20:2:1. Apoptotic cells were double-stained with FITC-anti-CD3 and PE-anti-Annexin V. The number of apoptotic T cells in the group incubated with HSCs was markedly greater than that in the group without HSCs (P<0.05).

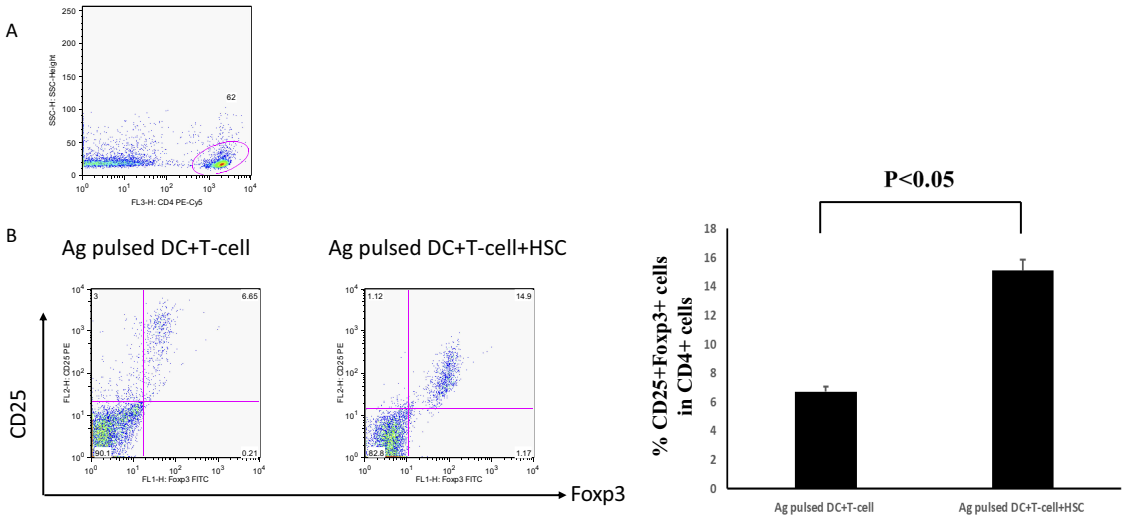

Figure 4 HSCs induced expansion of Treg cells. T cells were co-cultured with DCs in the presence or absence of HSCs. (**A**) Gated on the CD4+ cell populations. (**B**) HSCs increased the number of CD4+CD25+FoxP3+ cells, and the percentage of Treg cells in the group with HSCs was higher than that in the group without HSCs (P<0.05).

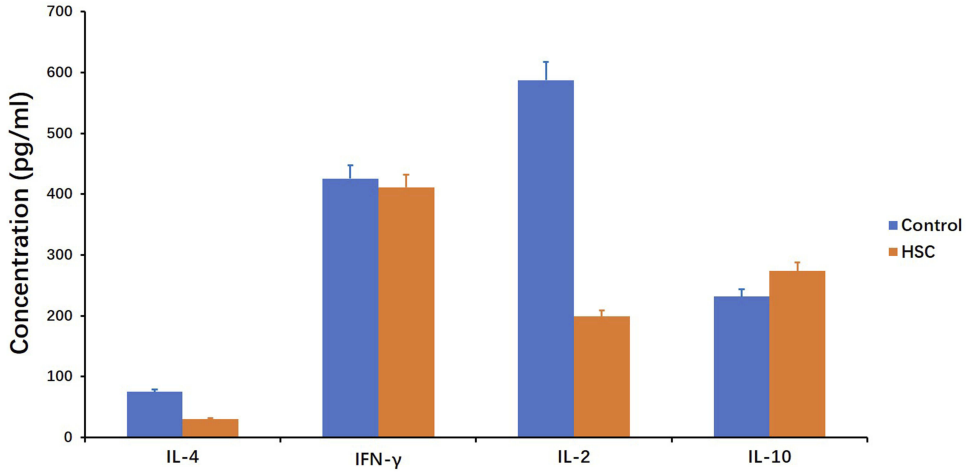

Figure 5 Supernatants were collected from MLRs and analyzed using ELISA. HSCs reduced the expression of IL-2 and IL-4 in the supernatants (P<0.05). However, HSCs did not alter the expression of IL-10 or IFN-γ in the supernatants (P>0.05).

proliferation and transwell migration assay was used to evaluate CT26 cell migration. As shown in Figure 6, supernatants from MLRs with HSCs promoted proliferation and migration of CT26 cells to a greater extent than MLRs without HSCs (P<0.05).

HSCs Promoted Growth Of Liver Metastatic Tumors In Vivo

To determine whether HSCs could promote CRC cell colonization in the liver in vivo, a murine model of CRC-derived liver metastasis was evaluated. In the control group, CT26 cells were injected via the portal vein. In the experimental group, a mixture of CT26 cells and HSCs was injected via the portal vein. As shown in Figure 7A, the number of liver metastatic tumors in the experimental group was greater than that in the control group (P<0.05). CT26 cell proliferation was assessed using PCNA immunostaining of liver metastatic tumor samples. The number of PCNA-positive cells was significantly greater in the experimental group than in the control group (P<0.05) (Figure 7B).

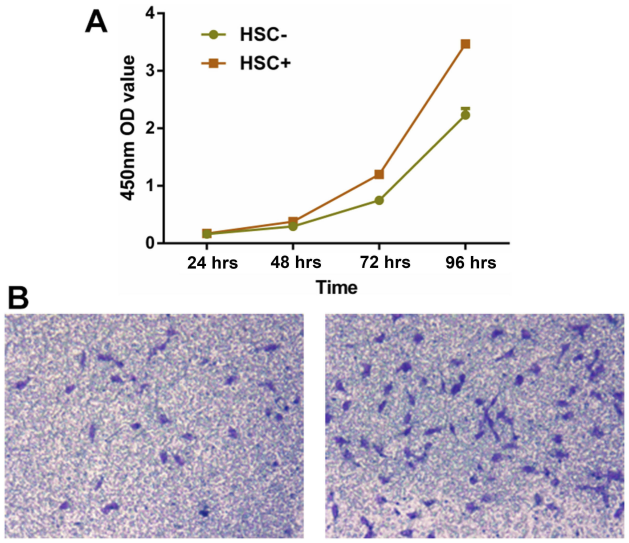

Figure 6 (**A**) CCK8 assay was used to determine whether supernatants of MLRs containing HSCs promoted proliferation of CT26 cells. (**B**) Transwell migration assay was used to determine whether supernatants of MLRs containing HSCs increased CT26 cell migration.

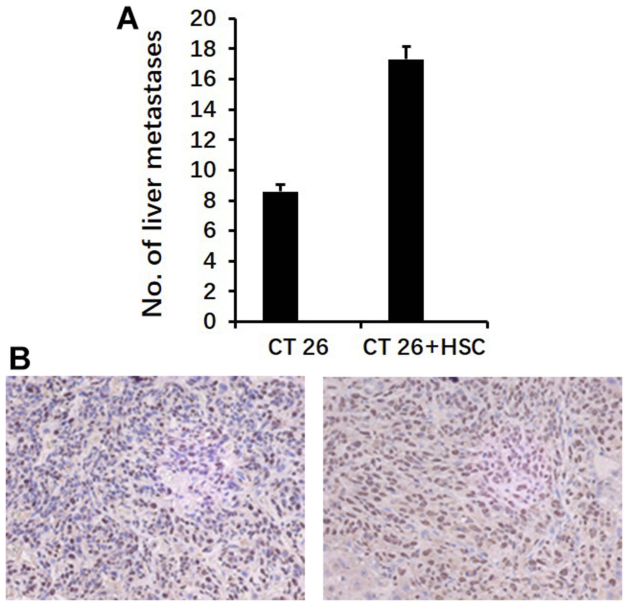

Figure 7 Effect of HSCs on liver metastasis in vivo. (**A**) The number of liver metastatic tumors in the experimental group was greater than that in the control group (P<0.05). (**B**) Immunohistochemical staining for PCNA in liver metastatic tumor samples. The number of PCNA-positive cells in the experimental group was significantly greater than that in the control group (P<0.05).

Discussion

CRC is a common malignancy of the gastrointestinal tract. The liver, which is nourished by a rich blood supply from both arterial and portal venous systems, is the most common location of CRC metastases. Liver metastasis is the main cause of CRC-related death.[14] A large body of evidence has indicated that the liver microenvironment provides autocrine and paracrine signals originating from both parenchymal and non-parenchymal cells that promote development of hepatic metastases.[15]

HSCs are the main non-parenchymal cells in the liver. Quiescent HSCs can be activated by cytokines or by liver injury. Recent studies showed that activated HSCs play an important role in liver fibrosis and exhibit immunomodulatory activities. Additionally, HSCs in the liver microenvironment have been shown to promote proliferation and infiltration of hepatoma cells.[16,17] Platelet-derived growth factor-C, which is secreted by colon carcinoma cells, induces proliferation of HSCs and promotes tumor growth.[18] However, the mechanisms responsible for the effects of HSCs on development of CRC-derived liver metastases remain unclear.

HSCs exert potent immunosuppressive effects via induction of activated T cell apoptosis.[19] Furthermore, HSCs can induce T cell hypo-responsiveness and promote Treg cell expansion, allowing hepatocellular carcinoma cells to escape immune detection, resulting in development of liver carcinoma.[20] We used MLRs to investigate the effects of HSCs on T cells. Our findings demonstrated that activated HSCs inhibited T cell proliferation and induced T cell apoptosis. In addition, HSCs promoted Treg cell expansion. Treg cells are a subgroup of CD4+ T cells that exhibit immunosuppressive activity. Transforming growth factor β (TGF-β) was one of the first cytokines discovered, and plays an important role in differentiation of Treg cells.[21] Activated HSCs secrete TGF-β,[11] which may have been responsible for the increased percentage of CD4+CD25+ FoxP3+ cells in MLRs with HSCs. Based on this result, we hypothesized that HSCs may be responsible for creating an immunosuppressive liver microenvironment, resulting in an environment suitable for the growth of CRC metastases.

We also analyzed cytokine levels in MLR supernatants. IL-2 is primarily secreted by CD4+ and CD8+ T cells, resulting in T cell activation and enhanced NK cell activity.[22] Consistent with our results, Shimizu et al[23] found that IL-2 production was significantly decreased in patients with CRC and hepatic metastasis. IL-4 is primarily secreted by type 2 T helper cells, mast cells, and basophilic granulocytes.[24] Recent studies have shown that binding of IL-4 to IL-4R promotes tumor cell proliferation in breast and prostate cancer.[25,26] However, Morisaki et al[27] found that IL-4/IL-4R prevents gastric cancer cells from entering the G0/G1 phase, resulting in inhibition of cell proliferation. Our results suggest that IL-4 inhibits metastasis of CRC to the liver.

MLRs were used to simulate an immune microenvironment. HSCs, which are liver non-parenchymal cells, were added to MLR cultures to create a hepatic microenvironment in vitro. CCK8 and transwell migration assays showed that supernatants from MLRs containing HSCs promoted CT26 cell proliferation and migration, confirming that HSCs in hepatic microenvironments could induce CRC cell proliferation and migration. Consistent with our in vitro results, we found that the presence of HSCs increased the number of liver metastases and increased proliferation of liver metastatic tumor cells in vivo.

Conclusion

Our study showed that HSCs may contribute significantly to an immunosuppressive hepatic microenvironment, resulting in increased CRC cell proliferation and migration. Furthermore, HSCs promoted CRC cell colonization in the liver. Our findings indicate a potential strategy for treatment of CRC liver metastases.

Acknowledgment

This study was supported by the “Belt and Road” Young Scientist Communication International Cooperation Project (17410742100).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Aran V, Victorino AP, Thuler LC, Ferreira CG. Colorectal cancer: epidemiology, Disease mechanisms and interventions to reduce onset and mortality. *Clin Colorectal Cancer*. 2016;15:195–203. doi:10.1016/j.clcc.2016.02.008
2. Nakagawa K, Tanaka K, Nojiri K, et al. The modified glasgow prognostic score as a predictor of survival after hepatectomy for colorectal liver metastases. *Ann Surg Oncol*. 2014;21:1711–1718. doi:10.1245/s10434-013-3342-6
3. Zimmitti G, Shindoh J, Mise Y, et al. RAS mutations predict radiologic and pathologic response in patients treated with chemotherapy before resection of colorectal liver metastases. *Ann Surg Oncol*. 2015;22:834–842. doi:10.1245/s10434-014-4042-6

4. Kawada K, Hasegawa S, Murakami T, et al. Molecular mechanisms of liver metastasis. *Int J Clin Oncol*. 2011;16:464–472. doi:10.1007/s10147-011-0307-2
5. Ribatti D, Mangialardi G, Vacca A. Stephen paget and the 'seed and soil' theory of metastatic dissemination. *Clin Exp Med*. 2006;6:145–149. doi:10.1007/s10238-006-0117-4
6. Gu X, Wang Y, Xiang J, et al. Interferon- γ triggers hepatic stellate cell-mediated immune regulation through MEK/ERK signaling pathway. *Clin Dev Immunol*. 2013;2013:389807. doi:10.1155/2013/657424
7. Chou HS, Hsieh CC, Yang HR, et al. Hepatic stellate cells regulate immune response by way of induction of myeloid suppressor cells in mice. *Hepatology*. 2011;53:1007–1019. doi:10.1002/hep.24162
8. Yang HR, Chou HS, Gu X, et al. Mechanistic insights into immunomodulation by hepatic stellate cells in mice: a critical role of interferon-gamma signaling. *Hepatology*. 2009;50:1981–1991. doi:10.1002/hep.23202
9. Zhao W, Zhang L, Xu Y, et al. Hepatic stellate cells promote tumor progression by enhancement of immunosuppressive cells in an orthotopic liver tumor mouse model. *Lab Invest*. 2014;94:182–191. doi:10.1038/labinvest.2013.139
10. Xu Y, Zhao W, Xu J, et al. Activated hepatic stellate cells promote liver cancer by induction of myeloid-derived suppressor cells through cyclooxygenase-2. *Oncotarget*. 2016;7:8866–8878. doi:10.18632/oncotarget.6839
11. Yu MC, Chen CH, Liang X, et al. Inhibition of T-cell responses by hepatic stellate cells via B7-H1-mediated T-cell apoptosis in mice. *Hepatology*. 2004;40:1312–1321. doi:10.1002/hep.20488
12. Gu X, Xiang J, Yao Y, Chen Z. Effects of RNA interference on CD80 and CD86 expression in bone marrow-derived murine dendritic cells. *Scand J Immunol*. 2006;64:588–594. doi:10.1111/j.1365-3083.2006.01845.x
13. Kohashi S, Sato Y, Fukushima T, et al. Interferon-beta inhibits liver metastases from murine colon 26 carcinoma and its highly metastatic variant. *Surg Today*. 2007;37:474–481. doi:10.1007/s00595-006-3418-z
14. Kopetz S, Chang GJ, Overman MJ, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. *J Clin Oncol*. 2009;27:3677–3683. doi:10.1200/JCO.2008.20.5278
15. Milette S, Sicklick JK, Lowy AM, Brodt P. Molecular pathways: targeting the microenvironment of liver metastases. *Clin Cancer Res*. 2017;23:6390–6399. doi:10.1158/1078-0432.CCR-15-1636
16. Amann T, Bataille F, Spruss T, et al. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci*. 2009;100:646–653. doi:10.1111/j.1349-7006.2009.01087.x
17. Antoine M, Tag CG, Gressner AM, Hellerbrand C, Kiefer P. Expression of E-selectin ligand-1 (CFR/ESL-1) on hepatic stellate cells: implications for leukocyte extravasation and liver metastasis. *Oncol Rep*. 2009;21:357–362.
18. Bandapalli OR, Macher-Goeppinger S, Schirmacher P, Brand K. Paracrine signalling in colorectal liver metastases involving tumor cell-derived PDGF-C and hepatic stellate cell-derived PAK-2. *Clin Exp Metastasis*. 2012;29:409–417. doi:10.1007/s10585-012-9459-3
19. Su YH, Shu KH, Hu C, et al. Hepatic stellate cells attenuate the immune response in renal transplant recipients with chronic hepatitis. *Transplant Proc*. 2012;44:725–729. doi:10.1016/j.transproceed.2011.11.049
20. Zhao W, Su W, Kuang P, et al. The role of hepatic stellate cells in the regulation of T-cell function and the promotion of hepatocellular carcinoma. *Int J Oncol*. 2012;41:457–464. doi:10.3892/ijo.2012.1497
21. Chen W, Jin W, Hardegen N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med*. 2003;198:1875–1886. doi:10.1084/jem.20030152
22. Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH. IL-6, IL-18, sIL-2R, and TNFα proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. *J Affect Disord*. 2015;182:106–114. doi:10.1016/j.jad.2015.04.044
23. Shimizu H, Ito H, Kimura F, et al. Decreased cell-mediated immune status in colorectal cancer patients with hepatic metastasis. *Hepatogastroenterology*. 2005;52:1106–1109.
24. Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD. Interleukin-4 and interleukin-13 signaling connections maps. *Science*. 2003;300:1527–1528. doi:10.1126/science.1085458
25. Roca H, Craig MJ, Ying C, et al. IL-4 induces proliferation in prostate cancer PC3 cells under nutrient-depletion stress through the activation of the JNK-pathway and survivin up-regulation. *J Cell Biochem*. 2012;113:1569–1580. doi:10.1002/jcb.24025
26. Venmar KT, Kimmel DW, Cliffel DE, Fingleton B. IL4 receptor α mediates enhanced glucose and glutamine metabolism to support breast cancer growth. *Biochim Biophys Acta*. 2015;1853:1219–1228. doi:10.1016/j.bbamcr.2015.02.020
27. Morisaki T, Yuzuki DH, Lin RT, Foshag LJ, Morton DL, Hoon DS. Interleukin 4 receptor expression and growth inhibition of gastric carcinoma cells by interleukin 4. *Cancer Res*. 1992;52:6059–6065.