Therapeutic modulators of hepatic stellate cells for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of primary tumor in the liver and is a leading cause of cancer-related death worldwide. Activated hepatic stellate cells (HSCs) are key components of the HCC microenvironment and play an important role in the onset and progression of HCC through the secretion of growth factors and cytokines. Current treatment modalities that include chemotherapy, radiotherapy and ablation are able to activate HSCs and remodel the tumor microenvironment. Growing evidence has demonstrated that the complex interaction between activated HSCs and tumor cells can facilitate cancer chemoresistance and metastasis. Therefore, therapeutic targeting of activated HSCs has emerged as a promising strategy to improve treatment outcomes for HCC. This review summarizes the molecular mechanisms of HSC activation triggered by treatment modalities, the function of activated HSCs in HCC, as well as the crosstalk between tumor cells and activated HSCs. Pathways of activated HSC reduction are discussed, including inhibition, apoptosis, and reversion to the inactivated state. Finally, we outline the progress and challenges of therapeutic approaches targeting activated HSCs in the development of HCC treatment.

Introduction
Hepatocellular carcinoma (HCC) accounts for about 90% of primary liver cancers and is the second most lethal cancer worldwide.1,2 Despite significant advances in the treatment of HCC, patients still have poor prognosis and the relapse rate is over 50%.3,4 Increasing evidence has shown that the tumor microenvironment (TME) plays a vital role in HCC progression and therapeutic response. Therapeutic targeting of components of the TME may provide a novel approach to the treatment of HCC.5 Hepatocellular carcinoma (HCC) is the most common type of primary tumor in the liver and is a leading cause of cancer-related death worldwide.1,2 Despite significant advances in the treatment of HCC, patients still have poor prognosis and the relapse rate is over 50%.3,4 Increasing evidence has shown that the tumor microenvironment (TME) plays a vital role in HCC progression and therapeutic response. Therapeutic targeting of components of the TME may provide a novel approach to the treatment of HCC.5

Key words: hepatocellular carcinoma, therapeutic modulators

Abbreviations: Angs: angiopoietins; Arg-1: arginase 1; α-SMA: α-smooth muscle actin; BrMC: 8-bromo-7-methoxychrysin; CAF: cancer-associated fibroblast; COL1A1: collagen 1A1; COX2: cyclooxygenase 2; CTGF: connective tissue growth factor; CXCR4: C-X-C receptor type 4; ECM: extracellular matrix; ERK: extracellular signal-regulated kinase; FAK: focal adhesion kinase; FAP: fibroblast activation protein; Galectin-3; GDF15: growth differentiation factor 15; HCC: hepatocellular carcinoma; HGF: hepatocyte growth factor; Hh: hedgehog; HIF-1α: hypoxia-inducible factor 1 α; HSC: hepatic stellate cell; HVEC: hepatic vascular endothelial cell; IFNα: type 1 interferons; IL-4Rα: interleukin-4 receptor alpha; IL-6: interleukin-6; IL-8: interleukin-8; iNOS: inducible nitric oxide synthase; JAK: janus kinase; Ln: laminin; MAPK: mitogen-activated protein kinase; MBL: mannose-binding lectin; MDSC: myeloid-derived suppressor cell; MEK: mitogen-activated protein kinase kinase; Mig: monocyte chemotactic protein-1; Mip-1α: monocyte chemoattractant protein-1; MiR: microRNA; MMP: metalloproteinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; Nox4: NADPH oxidase 4; PDG: platelet-derived growth factor; PDGFRβ: platelet-derived growth factor receptor-beta; PI3K: phosphoinositide 3-kinase; PTCH1: patched 1; RFA: radiofrequency ablation; ROS: reactive oxygen species; SDF-1α: stromal-derived factor 1 α; STAT: signal transducer and activator of transcription proteins; TACE: transarterial chemoembolization; TGFβ: transforming growth factor β; TLR4: toll-like receptor 4; TME: tumor microenvironment; TNFα: tumor necrosis factor; Treg: regulatory T cell; VDR: vitamin D receptor; VEGF: vascular endothelial growth factor

DOI: 10.1002/ijc.32899

History: Received 6 Dec 2019; Accepted 21 Jan 2020; Online 3 Feb 2020
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smooth muscle actin (α-SMA) and become proliferative and contractile. Activated HSCs are a key component in the TME of HCC and exhibit biological functions that influence the onset and progression of HCC. Activated HSCs secrete extracellular matrix (ECM), cytokines and growth factors to create a tumor-favoring environment, promoting tumor development, metastasis, and chemoresistance. Currently, multitherapeutic approaches are used to treat advanced or unresectable HCCs, including radiotherapy, chemotherapy, and radiofrequency ablation (RFA). However, these therapeutic strategies have been reported to promote the activation of HSCs. Furthermore, there is a lack of a critical understanding of the specific mechanisms involved in HSC activation and the effects of activated HSCs in the progression and relapse of HCC.

In this review, we systematically summarize the molecular mechanisms of HSC activation, which is notably triggered by current treatment modalities. We discuss how activated HSCs remodel the TME and interact with tumor cells to further promote tumor proliferation, angiogenesis, metastasis and resistance to current therapies. We emphasize the strategies that inhibit HSC activation and induce HSC apoptosis by targeting different molecules in signaling pathways involved in the activation of HSCs in the progression and relapse of HCC.

Interaction Between HSCs and Tumor Cells
The interaction between cancer cells and HSCs is bidirectional. Tumor cells are able to promote HSC activation and proliferation through transforming growth factor β1 (TGF-β1) mediated pathway and secretion of growth differentiation factor 15 (GDF15). Meanwhile, activated HSCs in turn generate a variety of cytokines, growth factors and ECM proteins, which contribute to forming a microenvironment favorable for tumor growth and promote tumor cell proliferation, metastasis and chemotherapy resistance. Thus, the crosstalk between activated HSCs and HCC cells leads to transdifferentiation of HSCs into myofibroblast-like cells which nourish tumor cells. Figure 1 summarizes the paracrine mechanisms whereby activated HSCs promote HCC development and treatment resistance.

Promotion of tumor angiogenesis
Activated HSCs express numerous growth factors, including vascular endothelial growth factor (VEGF)-α, PDGFβ and angiopoietins (Angs), which can promote angiogenesis by binding to their cognate receptors on the surface of endothelial cells. Activated HSCs secrete Ang-1 to enhance hepatic vascular endothelial cell (HVEC) growth and induce microtubule formation of HVEC in vitro. In addition to growth factors, activated HSCs also release proinflammatory chemokines that are able to stimulate angiogenesis. For example, IL-8 released from HSC promotes HCC angiogenesis by activating the STAT3 signaling pathway in hepatoma cells. Furthermore, under hypoxic conditions in the TME, HIF-1 is prevented from ubiquitination and activates VEGF transcription resulting in elevated VEGF production. The role of VEGF is further evidenced by experiments in immunocompetent mice where co-transplantation of HSCs and tumor cells exhibited higher microvessel density compared to transplantation of tumor cells alone. Thus, both in vitro and in vivo data suggest that activated HSCs play a proangiogenic role in HCC.

Promotion of HCC progression
Activated HSCs can promote tumor growth in HCC by paracrine release of hepatocyte growth factor (HGF), TGF-β1 and IL-6. Mechanistically, HCCs express c-Met, a receptor of HGF and binding of HGF to c-Met activates both extracellular signal-regulated kinase (ERK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways that promote carcinogenesis in HCC. Furthermore, culturing...
HCCs with conditioned medium of activated HSCs, enhanced proliferation and migration of HCCs through ERK and NF-κB signaling pathways. TGF-β1 derived from activated HSCs altered tumor cell behavior and tumorigenesis, resulting in tumor development and autocrine TGF-β signaling in tumor cells. TGF-β1 meditated tumor progression relies on Ras-ERK/MAPK signaling which accelerates the cell cycle and prevents apoptosis of the malignant hepatocyte. Activated HSCs secrete IL-6 that drives tumor growth through STAT3 signaling, with a synergistic effect of IL-6 and CTGF released by HCC cells. Co-inoculation of primary HSCs with tumor cells in vivo leads to enhanced tumor growth and proliferation.

Activated HSCs are able to secrete matrix metalloproteinases (MMPs) to remodel ECM around the tumor. As MMPs play a key role in degrading basement membranes, cancer cells are able to cross tissue boundaries after MMP upregulation. Evidence suggests that either conditioned media of activated HSCs or coculture with activated HSCs induces migration and invasion of HCC cells in vitro, mainly due to the increased MMP9 derived from activated HSCs. The number of activated HSCs is associated with tumor invasion of the portal vein and is dependent on focal adhesion kinase (FAK)-MMP9 signaling. In mixed cell spheroids of activated HSCs and HCC cells, epithelial contacts are altered by activated HSCs, which is mediated by decreasing E-cadherin and increasing vimentin expression. Both E-cadherin and vimentin are epithelial–mesenchymal transition (EMT) markers, suggesting activated HSCs may induce EMT-like phenotypic changes in HCC cells by upregulation of TGF-β and CTGF. Activated HSCs also promoted transglutaminase 2 (TGM2) upregulation in HCC cells, creating a pseudohypoxic environment that induced EMT in HCC cells. Furthermore, a 3D matrix invasion assay showed that invasive migration of tumor cells followed the same movement path of fibroblasts, indicating that fibroblasts may also secrete chemokines to attract tumor cells. Taken together, activated HSCs contribute to a reactive stroma that favors tumor invasion and migration.

Promotion of HCC chemoresistance
In the presence of activated HSCs, tumor cells appear to be resistant to chemotherapeutic agents mainly due to excessive ECM around the tumor and cytokines, both of which are derived from activated HSCs. An in vitro model showed that mixed cell spheroids of HSC and HCC were less sensitive to chemotherapeutics compared to spheroids containing HCC cells alone. It has also been suggested that HSCs facilitate chemoresistance of HCC cells in mixed cell spheroids to sorafenib and cisplatin by increasing collagen 1A1 (COL1A1) expression to generate a more compacted spheroid. Another study using a similar 3D cell culture model showed that activated HSCs express more profibrotic factors such as TGF-β1 and CTGF, resulting in the chemoresistance of HCC cells. Furthermore, laminin (Ln)-332, an ECM protein, is secreted abundantly by HSCs. When Ln-332 binds to α3β1 integrin, a receptor on HCC cells, it is able to prevent fibroblast activation protein (FAP) from ubiquitination which is a mechanism underlying sorafenib-induced apoptosis. Hence, activated HSCs enhance HCC chemoresistance by decreasing drug uptake and drug-induced apoptosis.

Suppression of the antitumor immune response
Activated HSCs in HCC not only reduce the number and function of T cells, but also promote the accumulation of immunosuppressive cells. B7 homolog 1 (B7H1/CD274/PD-L1) has emerged as an important regulator of host immune suppression. Upregulation of B7H1 on activated HSCs in the TME is a key mechanism through which tumor cells can escape the host immune system, including inhibiting T-cell responses, inducing T-cell apoptosis, attenuating T-cell infiltration and suppressing T-cell-mediated cytotoxicity both in vitro and in vivo. Furthermore, a reduction of antitumor responses is also found in the whole immune system by co-transplantation of HSCs and HCC cells in immunocompetent mice. Co-transplantation of activated HSCs reduced both the CD3+ CD4+ and CD3+ CD8+ T-cell subtypes in spleens of immunocompetent mice carrying HCC derived tumors. Activated HSCs also induce accumulation of two main suppressive immune cell populations, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), in the spleen, bone marrow and tumor tissues. Accumulation of MDSCs inhibits T-cell proliferation by elevating expression of Arginase 1 (Arg-1), inducible nitric oxide synthase (iNOS) and interleukin-4 receptor alpha (IL-4Rα). Therefore, activated HSCs not only impact the immune response in the TME, but also participate in the maintenance of immune tolerance.

Molecular Mechanisms of HSC Activation by Anticancer Treatment
Since residual tumor and surrounding tissue are also exposed to therapy used to treat HCC, paracrine signals from cancer cells and other cell populations within the TME can initiate intracellular signaling within HSCs. Growing evidence shows that some anticancer treatment such as RFA, chemotherapy and radiotherapy can activate HSCs in the residual tumor to influence HCC development and therapeutic response. The key molecular mechanisms involved in HSC activation by therapeutic treatments are summarized in Figure 2. In fact, these mechanisms often regulate, converge or intersect with other pathways to form an interconnected signaling network of HSC activation.

Chemotherapy-induced HSC activation
According to current clinical practice guidelines, patients with advanced-stage HCC are candidates for chemotherapy such as transarterial chemoembolization (TACE). TACE can deliver a higher concentration of chemotherapeutic drugs in the tumor followed by intra-arterial injection of an embolic agent, which induces ischemia and increases retention time of chemotherapeutic agents in the tumor. However, the long-term survival of HCC patients treated with TACE remains unsatisfactory due to
The molecular mechanisms of hepatic stellate cell (HSC) activation by anticancer treatment for hepatocellular carcinoma (HCC). Thermal ablation induces HSC activation through inflammatory cytokine, interleukin-6 and by activating both STAT3 and mitogen-activated protein kinase (MAPK) signaling pathways. Molecular inhibitors such as sorafenib promote HSC activation through the MAPK signaling pathway. Under the condition of hypoxia, both molecular inhibitors and chemotherapy can cause activation of stromal-derived factor 1 and hypoxia-inducible factor 1α, resulting in HSC activation after binding C-X-C receptor type 4 on HCCs. Transforming growth factor β is a key signaling pathway mediating HSC activation after chemotherapy or radiotherapy. The increased expression level of Nox4 subsequently induces translocation of transcriptional factors, Smad2/3, leading to HSC activation. Radiotherapy also promotes secretion of Hh ligands which bind to SMO on the HSC membrane resulting in HSC activation via Gli1/2 transcription factors.

Furthermore, chemotherapeutic treatment induced a high level of expression of GDF15 in HCC cells in vitro, promoting the proliferation and activation of HSCs through ERK1/2- and Smad3-dependent pathways.66

**Molecular inhibitor-induced HSC activation**

Sorafenib is a broad kinase inhibitor, and is a standard therapy for HCC which has been shown to exert antifibrotic effects in liver cirrhosis.51 However, sorafenib was found to induce HSC myofibroblast differentiation by activating the mitogen-activated protein kinase (MAPK) pathway in HSCs.54 Sorafenib can also enhance tumor hypoxia, increasing stromal-derived factor 1 alpha (SDF-1α) expression in HCC. The SDF-1-α/C-X-C receptor type 4 (CXCR4) pathway directly promotes HSC activation through the MAPK pathway and via myeloid differentiation antigen-positive (Gr-1+) myeloid cell infiltration.52

**Radiation-induced HSC activation**

Radiotherapy has emerged as an effective treatment for intermediate-stage HCC,10 but classic radiotherapy has mostly failed due to the side effects of radiotoxicity on the surrounding tissues.12 Radiation-induced liver disease, including late fibrosis, has been considered as a major limitation of radiotherapy for liver cancer.10,41 HSCs are highly radiosensitive cells, which are activated and accumulate in the livers of patients after radiotherapy.55 The activation of HSCs by radiation is a key cellular process underlying hepatic fibrosis, which can promote radioreistance and tumor recurrence.12 Furthermore, consistent results have also been observed in rats and mice, where the expression of α-SMA, a marker of HSC activation, was significantly increased in irradiated livers.36–38

TGF-β signaling is upregulated by radiotherapy and is a key regulator of HSC activation in the TME.12 TGF-β activated by radiation promotes the production of profibrotic cytokines and stimulates HSCs, by overproduction of plasminogen activator inhibitor-1, one of the downstream targets of TGF-β.59 In irradiated rat livers, the level of TGF-β1 increased with increasing dose of radiation and the expression of TGF-β1 correlated with the degree of hepatic fibrosis.60 The alteration in mRNA expression of TGF-β1 and TGF-β3 occurred at a very early stage after radiation in rat liver.61 Recent evidence also suggests that free radicals produced after exposure to radiotherapy may directly activate TGF-β1.51,59 Furthermore, the stimulated TGF-β1 could prolong the production of ROS in surrounding cells such as hepatocytes.59

Emerging evidence suggests that Hedgehog (Hh) signaling is also critical to radioreistance of HCC and regulates HSC activation induced by radiation.12 In HCC, activation of Hh signaling is associated with increased radiation resistance and decreased cell death.77 Irradiation of HCC cells induced the release of sonic Hh ligand and activated sonic Hh signaling with upregulation of sonic Hh, protein patched homolog 1 (PTCH-1) and Gli-1 expression, which could protect HCCs against ionizing radiation.62 The Hh pathway is activated in nontumor liver tissues after irradiation where both Smoothened (Hh receptor) and Gli2
Radiofrequency ablation-induced HSC activation

RFA is increasingly used to treat HCC patients, with its recent incorporation into clinical practice algorithms. However, increasing studies indicate that ablation could stimulate residual tumor growth and cause tumor recurrence.13 Hepatic RFA induces a local inflammatory zone with a time-dependent immunologic response at the perimeter of a necrotic zone. Interleukin-6 (IL-6) levels are elevated in the serum of mice after RFA and a massive accumulation and migration of activated HSCs was found in the necrotic zone.66 Inflammatory cytokines, including IL-6, interleukin-8 (IL-8), tumor necrosis factor (TNFα) and type I interferons (IFNα), have been shown to regulate HSC activation by modulating NF-κB activity or the Janus kinase (JAK)-signal transducer and activator of transcription proteins (STATs) signaling pathway.49 Knockout of IL-6 results in a reduction of activated HSCs in the border zone of RFA, suggesting that HSCs are activated by cytokine-mediated signaling after RFA in the liver. A similar result was found in HCC after incomplete RFA, where accumulation of activated HSCs was observed in surrounding residual HCC cells.13 Furthermore, hepatic RFA can increase the expression of cyclooxygenase-2 (COX-2) receptor in the liver, which is linked to the RFA-induced inflammatory pathway. Inhibition of COX-2 after hepatic RFA can suppress periablational cellular infiltration and inflammation-mediated HSC recruitment and activation.55

Overall, chemotherapeutic agents and molecular inhibitors exert their effect on the activation of HSCs mainly through the TGF-β, hypoxia and MAPK mediated signaling pathways. Hh- and TGFβ-mediated molecular signaling pathways regulate HSC activation induced by radiation and are critical to radioreistance of HCC, while RFA induces HSC activation mainly through an inflammatory cytokine-mediated pathways (Fig. 2).

Therapeutic Targeting of HSCs

The inhibition of HSC activation and control of HSC functions represent a promising therapy in HCC by focusing on developing targeting strategies for activated HSCs, including inhibition of HSC activation, induction of HSC apoptosis, reversion of HSCs to quiescent status and regulation of the interactions between HSCs and tumor cells.67–69 Below we discuss some promising targeting therapies currently being investigated and summarize targeting of signaling pathways involved in HSC transformation (Table 1).

TGF-β pathway targeting

As stated above, TGF-β is a critical cytokine regulating HSC activation processes, and links many factors that can lead to HSC transformation such as ROS, hypoxia and radiotherapy.70–72 Hence, most strategies to inhibit HSC activation are through targeting the TGF-β signaling pathway.66 When TGF-β binds to and phosphorylates its receptor on the cell membrane, SMAD2 and SMAD3 are phosphorylated and become active. They then bind to SMAD4 and translocate into the nucleus and activate the transcription of target genes such as α-SMA, resulting in HSC activation.70,74 Three main approaches have been applied to target the TGF-β pathway: (i) blocking the binding or translocation of SMAD proteins; (ii) decreasing the expression of TGF-β1; (iii) downregulating the expression of SMAD proteins.

Drugs used to treat liver fibrosis and cirrhosis have been considered for either inhibiting HSC activation or killing activated HSCs in cancer.67,73,75,76 Magnesium isoglycyrrhizinate (MgIG), a hepatoprotective drug for preventing and treating liver fibrosis, blocks the binding of SMAD2/3 and SMAD4 thereby suppressing the translocation of SMAD proteins to nucleus and inhibiting HSC activation.67 In the meantime, MgIG has been shown to induce apoptosis of activated HSCs by promoting endoplasmic reticulum stress, alleviating liver injury and fibrosis in carbon tetrachloride-induced liver fibrosis in mice.68 MgIG also exhibited a protective effect in oxaliplatin-induced liver injury by inhibiting oxidative stress and the IL-6 pathway.77 A molecule derived from maleic acid, designated compound 2, has multiple functions in inhibiting HSC activation to attenuate liver fibrosis. Compound 2 not only downregulates TGF-β downstream molecules, SMAD2, SMAD3 and transcription factor Sp1, but also decreases the ROS and Ca2+ levels in HSCs.79–80 GR-MD-02 and GM-CT-01 are galectin inhibitors, which have been used to treat liver fibrosis in clinical trials.79 HSC activation can be attenuated by both GR-MD-02 and GM-CT-01 through decreasing the expression of TGF-β1 in fibrotic livers.78 These drugs can be further considered as a possible combined treatment with conventional therapeutic agents to reverse or inhibit the HSC activation process to improve the antitumor activity.

Some compounds have also been explored as potential therapeutics to inhibit the functions of activated HSC. Vitamin D receptor (VDR) is highly expressed in quiescent HSCs, but its expression is decreased by up to 40% during HSC activation.79 Using a mouse model of liver injury, administration of a VDR agonist, calcipotriol, nearly completely abrogated the fibrogenic response and reduced collagen deposition and fibrotic gene expression.80 Calcipotriol was shown to antagonize SMAD3/ TGFβ1 activation of profibrotic genes by reducing SMAD3 occupancy at coregulated genes thereby inhibiting TGF-β1 signaling.80 Similar results on stellate cell activity were found in pancreatic cancer where activated pancreatic stellate cells reversed to a quiescent form after treatment with calcipotriol, promoting chemotherapy efficacy.81 1β-Glycyrrhetinic acid (GA) is an important bioactive compound of licorice root and is able to attenuate HSC activation by decreasing the expression of SMAD3.82 In addition, GA inhibits HSC proliferation and induces HSC apoptosis via impairment of NF-κB DNA binding activity.83 Furthermore, GA attenuates the immunosuppression effect mediated by activated HSC by reducing T-cell apoptosis and Treg cell expression.69
MicroRNAs (MiRs) have recently been identified as novel agents for targeting HSCs.73,84 Aberrant expression of MiR-34 is involved in the progression of HCC and MiR-34a is decreased in HCC patients.73,84 A recent study demonstrated that MiR-34a-5p could directly target SMAD4 to deactivate the TGF-β1/SMAD3 pathway and inhibit HSC activation.69 MiR-146a-5p is able to attenuate HSC activation through regulating the phosphorylation level of SMAD2 and JNK in HSCs.85

MAPK pathway targeting
MAPK is a key pathway involved in the activation of HSCs by molecular inhibitors.54 There is a convergence of between MAPK and TGF-β signaling pathways for HSC activation, where molecules involved in MAPK signaling pathway, Ras, Raf-1, Mitogen-activated protein kinase kinase (MEK) and MAPK p42 and p44, can also be activated by TGF-β1.86 Transdifferentiation of HSCs can also be initiated by proinflammatory IL-6 and through both MAPK and JAK/STAT signaling pathways by increasing the phosphorylation level of p28, MAPK and STAT3.87

Delivery systems that target activated HSCs with specific drugs have also proven efficient in modifying HSC activity. AZD6244, a MEK inhibitor, and sorafenib were loaded into a nanoparticle to reduce HSC activation by inhibiting MAPK and NF-κB.54 To increase the targeting specificity, the nanoparticle was modified to target CXCR4 which is highly expressed on activated HSCs. Sorafenib/MEK inhibitor-loaded CXCR4-targeted nanoparticles showed antifibrotic effects, reduced primary HCC tumor formation and increased drug uptake.54 After systemic administration of this nanoparticle, the incidence of liver metastasis was suppressed in a spontaneous liver metastasis mouse model which was developed by inoculation of pancreatic ductal adenocarcinoma cells into the pancreas of FVB mice.54 As the SDF-1α/CXCR4 pathway directly promotes HSC activation through the MAPK pathway, the inhibitor of CXCR4 (AMD3100) reduced

| Drug developed | Target molecule | Mechanisms | Outcome of HSC/CAF |
|---------------|----------------|------------|--------------------|
| TGF-β pathway | MgIG | SMAD2/3, Bcl2 and Bax | Block SMAD2/3 translocation to nucleus and activate the caspase cascades | Transfer to a senescent state and induce apoptosis67,68 |
| Compound 2 | SMAD2/3 and Sp1 | Downregulate TGF-β downstream molecules to regulate the expression of Col1A2 | Inhibit HSC activation75 |
| GR-MD-02/GM-CT-01 | Galecin | Decrease expression of TGF-β1 | Inhibit HSC activation76 |
| Calciopretilol | VDR | VDR/SAMAD3 genomic competition | Inhibit HSC activation80 |
| 18β-GA | SMAD3 | Reduce the mRNA expression of SMAD3, COL1A2 and COL3A1 | Inhibit HSC activation and induce apoptosis82 |
| MicroRNA-34a-5p | SMAD4 | Downregulate TGF-β | Inhibit HSC activation69 |
| MicroRNA-146a-5p | IRAK1, TRAF6 | Inhibit TLR4/MyD88 pathway and regulate SMAD2 phosphorylation | Inhibit irradiation induced HSC activation85 |
| MAPK pathway | AZD6244 | MEK | CXCR4 targeting nanoparticles delivering inhibitors of MAPK signaling pathway | Inhibit HSC activation54 |
| AMD3100 | CXCR4 | Prevent effects of SDF-1α induced by sorafenib | Inhibit HSC activation53 |
| MBL | ERK phosphorylation | ERK-mediated COX-2/PEG2 alteration downregulates HSC activation profile | Inhibit HSC activation88 |
| FAP | Dox/PL-rGO nanosheet | FAP | Specific targeting of activated HSCs | Increase drug delivery89 |
| PT-100 | FAP | Reduce the cytokines released from tumor cells | Inhibit accumulation of CAFs90 |
| FAP vaccine | FAP | Boost CD8+ T cell population to kill CAFs | Directly kill CAFs92 |
| Other approaches | BGB324 | Ax1/AKT phosphorylation | Target Gas6/Ax1 axis | Inhibit HSC activation93 |
| JSI-124 and BrMC | Inhibitor of STAT3 | Inhibition of STAT3 activation | Block the interaction between tumor cells and HSCs96 |
| Trichostatin A | Inhibitor of histone deacetylase | Epigenetic modulation | Block the interaction between tumor cells and HSCs95 |

Table 1. Potential therapeutic strategies for HCC that target HSC activation

Abbreviations: 18β-GA, 18β-glycyrrhetinic acid; BrMC, 8-bromo-7-methoxychrysin; CAF, cancer-associated fibroblast; Col1A2, collagen 1A2; COX-2, cyclooxygenase 2; CXCR4, C-X-C receptor type 4; FAP, fibroblast activation protein; HSC, hepatic stellate cell; MAPK, mitogen-activated protein kinase; MBL, mannose-binding lectin; MEK, mitogen-activated protein kinase kinase; MgIG, magnesium isoglycyrrhizinate; SDF-1α, stromal-derived factor 1; STAT3, signal transducer and activator of transcription proteins; TGF-β, transforming growth factor β; TLR4, toll-like receptor 4; VDR, Vitamin D receptor.
HSC activation via blocking SDF-1α/CXCR4 and inhibiting the MAPK pathway. The treatment efficacy of sorafenib can be improved when combined with AMD3100 by reducing hypoxia-mediated HCC desmoplasia and inhibiting HCC growth in an orthotopic HCC model with underlying liver fibrosis in mice. Mannose Binding Lectin (MBL) has recently been shown to be involved in the innate immune response that can inhibit HSC transformation. MBL binds to the surface of LX-2 cells (human HSC cell line) and reduces ERK phosphorylation in LX-2 cells, leading to an alteration of ERK-mediated COX-2/PEG2 expression.

**FAP targeting on activated HSCs**

As FAP is selectively overexpressed on cancer-associated fibroblasts (CAFs) in the tumor microenvironment, specific targeting of FAP in HSCs may provide new opportunities to suppress the activity and activation of HSCs. A nanosheet was developed to achieve targeting delivery of therapeutic agents to CAFs, which contained FAP-cleavable peptide sequences that can be activated by FAP protease in CAFs, liberating the pore-forming peptide melittin. Doxorubicin was loaded in the center of the nanosheet. The nanosheet can be selectively cleaved to pore-forming melittin by FAP expressed on CAFs, the diffused melittin can form pores in tumor cells and CAFs, increasing the uptake of doxorubicin in tumor cells and CAFs to enhance antitumor efficacy. The tumor volumes markedly decreased after this treatment in tumor-bearing mice. Another FAP-targeting drug, PT100, is now in clinical trials for colon carcinoma. PT100 inhibits the accumulation of CAFs, enhances the response of tumors to chemotherapy, reduces the expression of cytokines and decreases the recruitment of inflammatory cells such as macrophages and dendritic cells. Therefore, it is promising for specific delivery by targeting FAP on activated HSCs and combined with chemotherapy for HCC treatment.

An oral DNA vaccine has been designed and constructed to target FAP of CAFs, which can kill CAFs through a CD8+ T-cell-mediated antitumor immune response. FAP-vaccinated mice showed a decrease of collagen type I expression, 70% greater uptake of chemotherapeutic drugs, suppression of tumor growth as well as a threefold prolongation in lifespan. Hence, stimulation of a specific immune response to activated HSCs in the TME is a promising new approach in HCC treatment.

**Targeting of other pathways**

Other strategies have also been developed for inhibiting the activity of activated HSCs by targeting other signaling pathways, blocking the interaction between HSCs and tumor cells, and epigenetic modification. For example, GGB324 is an inhibitor of Ax1, which was found to reduce HSC activation by decreasing Ax1/AKT phosphorylation and then disrupting Gas6/AX1 in vitro and in vivo. 8-Bromo-7-methoxychrysin (BrMC) and cucurbitacin I (JSI-124), a selective inhibitor of STAT3, synergistically attenuate the crosstalk between LX-2 and HCC cancer stem-like cells through inhibiting STAT3 activation. There is also an epigenetic modulation proposed, by Coulouarn et al., that histone deacetylase inhibitor trichostatin A could reverse the cross-talk between hepatoma cells and activated HSCs, thus affecting migration and angiogenic activity of HCC.

**Conclusions and Perspectives**

Therapeutic modulation of activation and functions of HSCs has become a critical strategy for anticancer treatment against HCC. Understanding the molecular mechanisms of HSC activation, which is notably triggered by current treatment modalities, will provide guidance to develop effective therapeutic HSC targeting combined with individual treatment modality. Some drugs that have been already used to treat liver fibrosis and cirrhosis are also ideal inhibitors of HSC activation and control HSC functions in HCC treatment. In addition to targeting key pathways that mediate HSC activation, other HSC-based therapeutic strategies are potential therapeutic strategies for the treatment of HCC, including stimulation of specific immune responses against activated HSCs, blocking the interaction between activated HSCs and tumor cells, as well as targeted drug delivery for activated HSCs. Furthermore, elucidating the epigenetic regulation of gene expression in HSC activation may lead to new directions for therapeutic agent development such as novel compounds that target histone methylation or acetylation to inhibit activated HSCs.

**Acknowledgements**

The study was supported by grant from National Health and Medical Research Council of Australia (APP1125794) and Gallipoli Medical Research Foundation. This research was carried out at the Translational Research Institute, Woolloongabba, QLD 4102, Australia. The Translational Research Institute is supported by a grant from the Australian Government. We acknowledge Mr Haotian Yang for his assistance in this review.

**Conflict of interest**

The authors declare that they have no competing interests.

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