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REVIEW

Targeting cancer stem cells in hepatocellular carcinoma

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Abstract: The poor outcome of patients with hepatocellular carcinoma (HCC) is attributed to recurrence of the disease after curative treatment and the resistance of HCC cells to conventional chemotherapy, which may be explained partly by the function of liver cancer stem cells (CSCs). Liver CSCs have emerged as an important therapeutic target against HCC. Numerous surface markers for liver CSCs have been identified, and include CD133, CD90, CD44, CD13, and epithelial cell adhesion molecules. These surface markers serve not only as tools for identifying and isolating liver CSCs but also as therapeutic targets for eradicating these cells. In studies of animal models and large-scale genomic analyses of human HCC samples, many signaling pathways observed in normal stem cells have been found to be altered in liver CSCs, which accounts for the stemness and aggressive behavior of these cells. Antibodies and small molecule inhibitors targeting the signaling pathways have been evaluated at different levels of preclinical and clinical development. Another strategy is to promote the differentiation of liver CSCs to less aggressive HCC that is sensitive to conventional chemotherapy. Disruption of the tumor niche essential for liver CSC homeostasis has become a novel strategy in cancer treatment. To overcome the challenges in developing treatment for liver CSCs, more research into the genetic makeup of patient tumors that respond to treatment may lead to more effective therapy. Standardization of HCC CSC tumor markers would be helpful for measuring the CSC response to these agents. Herein, we review the current strategies for developing treatment to eradicate liver CSCs and to improve the outcome for patients with HCC.

Keywords: cancer stem cell, therapeutic targets, surface maker, signaling pathways, transforming growth factor beta

Introduction

Hepatocellular carcinoma (HCC), a major cause of worldwide morbidity and mortality, is the third leading cause of cancer-related death.[1] Age-adjusted HCC incidence rates in the USA tripled between 1975 and 2005.[2] For early-stage disease, the curative treatment options include resection, radiofrequency ablation, and transplantation. For patients who have multifocal lesions in the liver without vascular invasion, transarterial chemoembolization has been shown to prolong survival. Treatment for patients with advanced HCC is limited. Sorafenib, a multikinase inhibitor, is the only systemic treatment approved by the US Food and Drug Administration that has conferred a modest survival benefit in this group by prolonging overall survival by 2 months in a randomized Phase III clinical trial.[3] However, because of recent advances in the molecular pathogenesis of HCC, specifically in our understanding of cancer stem cell (CSC) biology, there is potential for the development of many novel pharmacologic targets and therapeutic strategies.

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Liver cancer stem cells

A CSC concept was proposed several decades ago to explain two recurring observations, ie, that most cancers consist of phenotypically heterogeneous tumor cells resembling distinct stages of normal tissue development, and that only a fraction of cells from both hematologic and solid malignancies are tumorigenic.[4–6]

Accumulating evidence supports the origination of HCC from transformation of liver stem/progenitor cells. Approximately 40% of HCC cases have clonality and are thus believed to originate from progenitor/stem cells.[7–10] The liver has high regenerative potential, and small oval hepatic progenitor cells around the peripheral branches of the bile ducts, the canals of Hering, can differentiate into biliary epithelial cells and hepatocytes.[11] These oval liver progenitor cells share molecular markers with adult hepatocytes (albumin, cytokeratin 7, CK19, oval cell markers [OV6, A6, and OV1], chromogranin-A, and neural cell adhesion molecules) and fetal hepatocytes (α-fetoprotein).[7,11] They are also positive for more common stem cell markers such as CD34+, Thy-1+, c-Kit+, and FMS-like tyrosine kinase 3.[12]

The role of liver progenitor cells in hepatocarcinogenesis was first suggested in the Solt-Farber and diethylnitrosamine models of experimental liver injury and hepatocarcinogenesis in the rat.[13,14] Since then, more data have suggested that liver CSCs are responsible for cancer initiation, local recurrence, metastasis, and therapy resistance in subsets of HCC. In addition, microarray-generated molecular classifications of HCC suggest a potential stem cell origin in a subpopulation of human HCC and underline prognostic implications for the prospective analysis of putative CSCs.[15] Taken together, liver CSCs have emerged as the therapeutic target for HCC, and there is increasing interest in identifying strategies eradicating CSCs.[16,17]

CSC markers and potential therapeutic targets against liver CSCs

Numerous surface markers for HCC stem cells have been identified, and include CD133, CD90, CD44, CD13, and epithelial cell adhesion molecules (EpCAMs).[18–21] Although their roles in liver CSCs are not fully understood, studies have shown that targeting these markers can specifically inhibit liver CSCs with high efficacy. It is reported that the tumorigenicity and invasive capacity of liver CSCs can be impaired by targeting CSC surface markers, leading to reduction of the CSC pool.

A handful of new therapeutic agents have been developed to target CSC markers. VB4-845, an immune recombinant fusion protein targeting EpCAMs, has been shown to strongly suppress sphere formation and tumor formation in vivo by decreasing the cancer cell population expressing CD133 and CD13.[22] Targeting of the CD44 isoforms in HCC was able to selectively deplete CD44-positive HCC cells.[23] The anti-CD44 antibody-mediated liposomal nanoparticle has been developed to target CSCs and monitor cancer progression or regression in HCC cells.[24] 8-bromo-7-methoxychrysin, a synthetic analog of chrysin, has inhibited the proliferation, self-renewal, and invasion of liver CSCs in vitro and in vivo, downregulated the expression of liver CSC biomarkers CD133 and CD44, and induced epithelial-mesenchymal transition by downregulating the expression of Twist and β-catenin in liver CSCs.[25] Therefore, direct targeting of liver CSC-specific markers may be a promising therapeutic strategy for eradicating liver CSCs.

Stemness signaling that serves as a therapeutic target for HCC CSCs

One alternative approach to targeting CSCs has been to examine the cellular pathways that are required for regulation of normal stem cells. Many signaling pathways observed in normal stem cells can also be detected in CSCs. These pathways include but are not limited to Wnt/β-catenin, Hedgehog, and Notch signaling pathways.[26]

Disturbing the signaling involved in normal stem cell fate reportedly decreased the self-renewal and proliferating capabilities of CSCs. The signaling pathways, which are proposed to regulate liver CSC function and to contribute to the aggressive behavior of HCC, are often found to have the characteristics summarized in Figure 1. By reviewing the literature, we found that seven pathways have four to five of the properties described in Figure 1 that provide promising targets for treating HCC against liver CSCs.

Regulation of embryonic stem cell proliferation and differentiation.
Regulation of expression of stem cell markers.
Genetic alteration of the pathway promotes development of hepatocellular carcinoma in mice.
Alteration of the pathway increases tumor initiation capacity and/or chemoresistance in xenotransplant of hepatocellular carcinoma.

Figure 1 Properties of candidate signaling pathways that regulate liver cancer stem cell function.

Wnt signaling

The Wnt signaling pathways are involved in determining the proliferation and fate of embryonic and adult stem cells. The cascade has also been shown to regulate cell axis patterning and cell migration in the developing embryo. Dysregulation of this pathway is found in the carcinogenesis of multiple cancer types, including HCC. Liver-targeted disruption of adenomatous polyposis coli (APC) in mice activates β-catenin signaling and leads to HCC tumorigenesis.[27]

The Wnt pathway shares a common initiating event, a Wnt family protein binding to a transmembrane Frizzled G-protein coupled receptor (along with other requisite coreceptors and ligands), thereby activating the intracellular protein Dishevelled. The activated Frizzled-Dishevelled complex leads to intracellular accumulation of β-catenin by deactivating the ubiquitinating β-catenin destruction complex. High amounts of β-catenin then translocate into the nucleus, where they induce phenotypic changes through transcription factor activation.[28] Epithelial cell adhesion molecule (EpCAM), the downstream gene of Wnt signaling, forms a positive feedback loop in the promotion of liver CSC proliferation by activation of Wnt signaling. Upon proteolysis activation, EpCAM releases its intracellular domain, which translocates to the nucleus and associates with β-catenin and Lef-1 to transactivate genes and promote cell proliferation.[29] EpCAM-positive HCCs have been shown to have a poor prognosis and more aggressive behavior after xenotransplantation in nonobese diabetic/severe combined immunodeficient mice, thereby rendering EpCAM a putative marker of HCC CSCs.[29,30]

The Wnt pathway can be inhibited by blocking β-catenin interaction with the TCF gene; when this pathway was inhibited in one study, CSC numbers and spheroid formation were reduced.[31,32] These findings, showing the importance of the Wnt pathway in CSC viability, have led to a number of novel approaches to HCC treatment.[32–37] However, no clinical trials are under way for Wnt-specific small molecule inhibitors in HCC.

TGF-β signaling

Transforming growth factor beta (TGF-β) signaling pathways can either promote or inhibit tumorigenesis, depending on the cell microenvironment. TGF-β is excreted in an autocrine fashion in varying amounts, depending on the cell type. Activated TGF-β surface receptors II and/or III (TGFBII/III) form a complex with TGF-R1, which then activates the protein kinase intracellular domain of TGF-RI. This protein kinase activates Smad transcription factors 2 and 3, either of which then forms a complex with Smad 4 proteins, translocating to the nucleus to regulate gene expression.

A functional role of TGF-β signaling in liver stem cell niches has been demonstrated through mouse genetics. Disruption of TGF-β signaling yields a phenotype similar to that of a human CSC disorder, Beckwith-Wiedemann syndrome, a disease characterized by stem cell alteration.[38] Loss or reduced expression of the TGF-β receptor (TβRI/TβRII) or signaling molecules (eg, Smad 4) also enhances malignant progression in various human tumor types, cancer xenografts, and transgenic mice.[39–45] This is at least partially due to the activation of mitogenic and oncogenic pathways involving CDK4, PRAJA, β-catenin, TERT, and c-MYC, that occurs when the TGF-β pathway is inactivated.[46]

Disruption of TGF-β signaling by genetically removing one copy of Sptbn1, a Smad3 adapter protein, results in spontaneous development of HCC; expression analysis of these tumors highlighted marked activation of genes involved in the interleukin (IL)-6 signaling pathway, including IL-6 and Stat3.[10]

Chronic inflammation is a major risk factor for cancer development. The interaction between liver CSCs and surrounding immune cells plays an important role in hepatocarcinogenesis. Suppressed TGF-β signaling activates Toll-like receptor 4/NANOG, a mediator of the immune and inflammatory response that increases pluripotency genes and tumorigenesis and promotes the chemoresistance of liver CSCs.[47] In contrast, excessive TGF-β could increase expression of the CSC marker in HCC and render HCC highly tumorigenic after xenotransplantation.[48] Furthermore, when liver CSCs were exposed to TGF-β for long periods of time (similar to what occurs in a cirrhotic liver), they gained CSC properties, including increased marker expression, tumorigenicity, chemoresistance, and self-renewal capacity. A number of drugs targeting aberrant TGF-β signaling are in development and have shown promising preclinical activity in arresting growth and metastasis of HCC.[49–55] A TGF-β receptor tyrosine kinase inhibitor, Ly2157299, is currently being evaluated in HCC, with promising results.[56]

JAK-STAT

The Janus kinase (JAK)-STAT pathway is a unique intracellular signaling cascade that coactivates cytokine receptors after extracellular ligand binding, such as interferon, osteopontin, IL-6, and oncostatin. The binding of JAK protein to the intracellular domain of certain ligand-activated cytokine

receptors (most notably interferon receptors) initiates tyrosine transphosphorylation between JAK proteins and the intracellular domain of the cytokine receptor. The resulting phosphorylated tyrosine binds the SH2 domain of STAT proteins, causing their dimerization/activation. Dimerized STAT proteins can interact with other proteins within the cytoplasm or translocate to the nucleus to act as transcription factors. The JAK-STAT pathway has been shown to play a key role in hematopoiesis, differentiation, and clonal expansion of immune cells, and immune cytokine transduction in nonimmune cells.[57]

Mice with disruption of TGF-β signaling develop HCC and are found to have activation of IL-6/JAK/STAT3 signaling. Formation of HCC can be prevented by inhibition of IL-6/JAK/STAT3, indicating that IL-6/JAK/STAT3 plays an important role in the transformation of liver CSCs.[10,58] STAT3 inhibitors suppress the proliferation of HCC cells.[58] Recently, He et al reported that the liver progenitor cells are premalignant cells, and become malignant if they are transplanted in the damaged liver because transformation of these cells requires the correct microenviroment. These progenitor cells depend on autocrine IL-6 production and activation of IL-6/JAK/STAT3 during transformation in order to form HCC.[59] The IL-6/JAK/STAT3 signaling pathway may serve as a target for the prevention and treatment of HCC.

Notch

The Notch pathway plays an important role in cell-cell signaling, inducing proliferation, homeostasis, and/or differentiation, depending on cell lineage. The initiating cell expresses a variety of cell-bound proteins (termed DSL ligands) that bind to the extracellular domain of the target cell's transmembrane Notch family protein, inducing proteolytic cleavage of the intracellular and extracellular Notch components by γ-secretase and tumor necrosis factor-α component enzyme, respectively. The Notch intracellular domain is activated by further cleaving, translocates to the nucleus, and associates with CSL family DNA transcription factors to induce phenotypic changes in the target cell.[60]

Notch overexpression has been shown to be oncogenic in lung, breast, colon, and brain CSCs, and pharmacologic targeting of this pathway is already being studied in clinical trials for these cancers.[61–65] When the intracellular domain of Notch was conditionally expressed in liver progenitor cells, transgenic mice developed HCC within 12 months.[66,67]

Targeting the Notch pathway could allow inhibition of CSC self-renewal and a decrease in tumor growth. Inhibition of Notch signaling by γ-secretase inhibitors has decreased $EpCAM^+$ liver CSCs. Restored expression of the tumor suppressor gene RUNX3 has reduced CSCs in HCC by suppressing Jagged-1-Notch signaling.[66] Together, these findings suggest that Notch overactivation is involved in liver CSC in a significant subset of HCC populations, making it a valuable target for the development of therapeutics against HCC.

Hedgehog

The Hedgehog pathway is an important embryonic patterning cascade. Paracrine excretion of Hedgehog protein causes a variety of responses in the receiving cell, depending on the receiving cell type, concentration of Hedgehog protein, and concentration of the receiving cell's transmembrane receptor patched protein. Without Hedgehog binding, transmembrane receptor patched protein inhibits the transmembrane G-coupled receptor Smoothened. Hedgehog therefore activates Smoothened, which in turn activates zinc transcription factors in the GLI family through an incompletely understood mechanism. GLI proteins accumulate in the nucleus, activating or inhibiting Hedgehog gene targets.[68]

Chronic fibrosing liver injury is a major risk factor for hepatocarcinogenesis in humans. Mice with targeted deletion of Mdr2 (the murine ortholog of MDR3) develop chronic fibrosing liver injury and eventually HCC. Mdr2(−/−) mice consistently expressed Hedgehog ligands and progressively accumulated Hedgehog-responsive liver myofibroblasts and progenitors with age. Treatment of aged Mdr2-deficient mice with the Hedgehog signaling antagonist GDC-0449 significantly inhibited hepatic Hedgehog activity, decreased liver myofibroblasts and progenitors, reduced liver fibrosis, promoted regression of intrahepatic HCCs, and decreased the number of metastatic HCCs without increasing mortality.[69] Enhanced Hedgehog signaling activity was found to contribute to chemoresistance in HCC. A specific Hedgehog inhibitor, cyclopamine, not only significantly blocked Hedgehog signaling activity but also inhibited the proliferation of liver CSCs, suggesting that Hedgehog signaling is critical for the tumorigenicity of CSC subpopulations.

Many Hedgehog pathway inhibitors are being evaluated, and most agents focus on targeting the Smoothened. receptor.[70] Currently, agents targeting this pathway, including vismodegib (the most studied Smoothened inhibitor), are in preclinical and Phase I trials for HCC.

PI3K/AKT/mTOR and IGF1

The phosphatidylinositol-4 5-bisphosphate 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway plays an important role in cell proliferation and survival.

Activation of the PI3K/AKT/mTOR pathway has been shown to accelerate hepatocarcinogenesis driven by N-Ras or β-catenin.[71,72] Mutational inactivation of phosphatase and tensin homologue is found in a variety of cancers, including HCC.[73,74] Insulin growth-like factor/mTOR signaling has been shown to increase chemoresistance and tumor-imitating capacity in liver CSCs.[75–78] Small molecule inhibitors of this pathway have shown promise in a variety of cancers, including HCC.[79]

c-MET (hepatocyte growth factor receptor)

Another transmembrane receptor affecting multiple proliferative signaling pathways is the hepatocyte growth factor receptor, encoded by the c-MET oncogene. Hepatocyte growth factor receptor binds to the paracrine scatter factor, dimerizes, and transphosphorylates itself to create two catalytic tyrosine residues on its cytoplasmic domain. c-MET-positive cells demonstrated CSC-like characteristics, such as chemoresistance, tumor sphere formation, and increased expression of CD44 and ABCG2; furthermore, PHA665752, a c-MET inhibitor, suppressed tumor sphere formation and inhibited CD44 expression.[80] In addition, hepatocyte growth factor receptor released by stromal cells around liver CSCs were shown to be required for liver CSC homeostasis.[81] A variety of small molecule hepatocyte growth factor receptor/c-MET inhibitors are currently in development.[82]

Differentiation of liver CSCs

The differentiation of cancer cells into less aggressive forms has been a successful treatment strategy, as demonstrated in the treatment of acute promyelocytic leukemia. In one study, the application of all-trans retinoic acid after normal chemotherapy resulted in a 90% remission rate and a 70% cure rate in acute promyelocytic leukemia.[83] Therapeutic agents that promote the differentiation of liver CSCs have also been used to deplete these cells. Hepatocyte nuclear factor-1 alpha (HNF1α), one of the key transcription factors of the hepatocyte nuclear factor family, plays a critical role in hepatocyte differentiation. A recombinant adenovirus carrying the HNF1α gene inhibits HCC xenograft growth in mice by inducing the differentiation of hepatoma cells into mature hepatocytes and G(2)/M arrest.[84]

Oncostatin M, an IL-6-related cytokine known to induce the differentiation of hepatoblasts into hepatocytes, has been shown to induce the hepatocytic differentiation of EpCAM(+) HCC cells. EpCAM(+) HCC cells treated with oncostatin M have shown enhanced cell proliferation with expansion of the EpCAM-negative non-CSC population. A combination of oncostatin M and conventional chemotherapy with fluorouracil has efficiently eliminated HCC by targeting both CSCs and non-CSCs.[20] The identification of valid differentiation pathways in CSCs may lead to new therapeutic strategies against liver CSCs.

Targeting microRNAs in liver CSC

MicroRNAs (miRNAs) are a class of small non-coding RNAs involved in the transcriptional and post-transcriptional regulation of gene expression. Increasing evidence has demonstrated that miRNAs also play a critical role in normal liver development and in the fine-tuning of fundamental biological liver processes.[85] Studies of miRNAs as biomarkers and therapeutic targets in HCC have focused on the group of miRNAs maintaining the stemness of liver CSCs, and attribute the resistance of HCC to cytotoxic chemotherapy. The miRNA-181 family is highly expressed in EpCAM+ AFP+ cells. miRNA-181 cells exert their function by targeting Caudal type homeobox transcription factor 2, GATA binding protein 6, and Nemo-like kinase, which are essential for hepatic cell differentiation and the Wnt pathway.[85]

miRNA-130b is significantly elevated in CD133+ liver CSCs and is required for self-renewal, tumorigenicity, and chemoresistance.[86] miRNA-221 is another tumor-promoting miRNA. Overexpression of miRNA-221 in the liver results in spontaneous HCC development.[87] In contrast with miRNA-181 and miRNA-130b, miRNA-26a is a tumor suppressor miRNA. Expression of miR-26a was significantly downregulated in a MYC-induced hepatocarcinoma murine model (tet-o-MYC; LAP-tTA mice), and this result was further confirmed by detecting the expression profiling of miR-26a in human HCC and normal liver biopsy specimens.[88] The tumor suppressive role of miR-26a may be related to it's ability to regulate cell cycle progression by targeting cyclin D2 and cyclin E2, two influential players in G1/S cell phase transition. Ectopic expression of miRNA-26a results in blockage of cell proliferation and induction of tumor cell apoptosis. Furthermore, miRNAs are shown to regulate the drug sensitivity of HCC to chemotherapy. miRNA-21 is found to lower the sensitivity of HCC to chemotherapy by inhibiting phosphatase and tensin homologue or programmed cell death 4, but miRNA-101 sensitizes HCC cells to chemotherapy by inhibiting myeloid cell leukemia sequence 1, a well characterized antiapoptotic member of the Bcl-2 family.[89,90] Understanding the function of miRNAs in liver CSCs will provide a novel strategy to develop treatments against liver CSCs.

Table 1 Clinical trials evaluating novel therapeutics targeting candidate signaling pathways that regulate liver cancer stem cell function

Signaling pathway	Drug class(es)	Name/code	Trial phase	Cancer type	Major findings	Common G3/G4 AEs[2]	Notes
Wnt	Anti-Wnt2 Ab Λ secretase	–	–	–	–	–	Preclinical testing.[95]
Notch	Inhibitors Notch Abs	–	–	–	–	–	Preclinical testing.[96]
TGF-β	TGF-β KI	LY2157299[97]	II	HCC	mTTP 12 weeks, mOS 36 weeks.	None	AFP decline with tx correlated with mTTP.
	TGF-β Ab	PF-03446962[98]	I	HCC	mTTP 12 weeks.	Thrombocytopenia	–[98]
Hedgehog	SMO antagonist	GDC-0449[99–101]	I	ASTs	No DLTs.	Hyponatremia	Complicated PK, ongoing dosing trials pursued.[99]
JAK-STAT	JAK inhibitor	AZD1480[102]	I	ASTs	+DLTs, no tumor response.	Neuropsychiatric	Further study discontinued secondary to lack of tumor response, high 1st pass metabolism and neuropsychiatric AEs.
	STAT inhibitor	AZD9150[103]	I	ASTs	No response to tx seen.	Thrombocytopenia	One patient experienced TMA requiring emergent intervention.
		OPB-31121[104]	I	ASTs	No response to tx seen.	Unk	Phase I/II trial for HCC ongoing.
IGF1/mTOR	IGF-1R Ab	IMC-A12[105]	II	HCC	mOS 8 months. No response to tx seen.	Transaminitis, lymphopenia, hyperglycemia	–
		AVE1642[106]	I	HCC	Of 13 patients, eleven showed tumor stabilization at mean f/u time of 13.3 weeks.	Unk	Studied with concurrent sorafenib.
		BIIB022[107]	I	ASTs	Of 24 patients, 3 had objective improvement on PET scan.	None	No patient in this study had HCC.
		MEDI-573[108]	I	ASTs	12 week DCR 8/43 patients.	Unk	–
	IGF-1R KI	OSI-906[109]	I	ASTs	12 week DCR 24/61 patients.	Nausea, vomiting, diarrhea	Particularly strong activity against adrenocortical carcinoma.
	AKT inhibitor	MK2206[110]	I	ASTs	16 week DCR 3/33 patients.	Rash	DLTs (rash) experienced in majority of higher dosing regimens.
		Perifosine[111]	II	HCC	mTTP 14 weeks. 12 week DCR 15/32.	Fatigue, abdominal pain, transaminitis	Of 42 patients, only 32 were available for efficacy analysis.
	mTOR inhibitor	Sirolimus[112]	II	HCC	mTTP 15.3 weeks. mOS 26.4 weeks.	None	Two of 25 patients died from infections possibly due to study drug. (G5)
		Everolimus[113]	I/II	HCC	No DLTs. mTTP 3.8 months. mOS 8.4 months.	Transaminitis	–
		AZD8055	–	–	–	–	Phase I in progress.
		CC-223	–	–	–	–	Phase I in progress.
		Temsirolimus[114]	I	HCC	DCR17/25 patients at 8 weeks.	Hypophosphatemia, infection, thrombocytopenia, hand-foot skin reaction, fatigue	Studied in combination with sorafenib.
	PI3K inhibitor	BAY80-6946	–	–	–	–	Phase I in progress.
HGFR/c-MET	c-MET KI	Tivantinib[115]	II	HCC	mTTP 1.6 months vs 1.4 months placebo. In c-MET high tumors, 2.7 months vs 1.4 months placebo.	Neutropenia, anemia	–

	Cabozantinib[116]	II	HCC	mTTP 4.2 mo. DCR.	Diarrhea, hand-foot skin reaction, thrombocytopenia	–
	Crizotinib	–	–	–	–	Phase I in progress.
	Foretinib[117]	I	ASTs	Of 40 patients, 22 had stable disease at a mean f/u time of 4 months.	HTN, elevated LDH, proteinuria, fatigue, diarrhea, vomiting	G3/G4 reactions listed are for higher doses only.
	INC280	–	–	–	–	Phase I in progress.
	MSC2156119	–	–	–	–	Phase I in progress.
	c-MET Ab	Onartuzumab	–	–	–	–	Phase I in progress.
Other	Multikinase Inhibitor	Sorafenib[3]	II	HCC	Sorafenib: mTTP 5.5 months mOS 10.7 months. Placebo: mTTP 2.8 months mOS 7.9 months.	None	Only FDA-approved treatment for advanced HCC.

Abbreviations: Ab, antibody; G3/G4, grade 3/4; G5, grade 5; AEs, adverse events; mTTP, median time to progression; mOS, median overall survival; AFP, alfa fetoprotein; PK, pharmacokinetics; DLTs, dose-limiting toxicities; DCR, disease control rate (sum of disease response plus disease stability on treatment); FDA, US Food and Drug Administration; HTN, hypertension; LDH, lactate dehydrogenase; Tx, treatment; HCC, hepatocellular carcinoma; ASTs, advanced solid tumors; Unk, unknown (not enough information in publication); TMA, thrombotic microangiopathic anemia; TGF-β, transforming growth factor beta; SMO, Smoothened; JAK, Janus kinase; IGF, insulin-like growth factor; HGF, hepatocyte growth factor; mTOR, mammalian target of rapamycin; f/u, follow-up; PI3K, phosphatidylinositol-4 5-bisphosphate 3-kinase; c-MET, hepatocyte growth factor receptor.

Cancer stem cell niche

Disruption of the tumor niche that is essential for CSC homeostasis has become a novel strategy in cancer treatment.[91] As the main source of extracellular matrix proteins in the tumor stroma, hepatic stellate cells decrease the sensitization of HCC cells to chemotherapeutic agents by promoting epithelial-mesenchymal transition and CSC-like features via hepatocyte growth factor/Met signaling.[81,92] Apart from the molecular content surrounding liver CSCs, there is accumulating evidence that the physical environment is a critical mediator of HCC tumor behavior.[93] The stiffness of the matrix is a strong predictor of HCC development. Increasing stiffness was found to promote HCC cell proliferation.[93] In contrast, a soft environment induced reversible stem cell characteristics in HCC.[94]

Summary and future directions

Liver CSCs have emerged as a therapeutic target for HCC, resulting in a paradigm shift from traditional therapies to those aimed at eradicating CSCs. Strategies for eliminating liver CSCs have been developed to target CSC markers, signaling transduction pathways, and the CSC niche. Clinical trials using inhibitors of TGF-β signaling (LY2157299, PF-03446962), the Hedgehog pathway (GDC-0449, PF-04449913, BMS-833923, IPI-926, TAK-441), the Notch pathway (RO4929097, BMS-906024, MK0752), and Wnt pathways (PRI-724) have started to emerge, but their efficacy with regard to CSC function remains to be determined.[54] Table 1 shows current clinical trial progress in targeting embryonic stem cell pathways shown to be overexpressed in HCC CSCs. Challenges remain, including the expressive heterogeneity of these pathways between patient tumors, which render agents targeting a single pathway less effective against HCC. More study of the genetic makeup of patient tumors that respond to these small molecule inhibitors may lead to more effective therapy and stronger clinical trial outcomes. Standardization of HCC CSC tumor markers would be helpful for measuring the CSC response to these agents. The CSC theory is an exciting new paradigm that has shown promise for future therapy ex vivo. The translational effectiveness of targeting CSCs remains to be determined.

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Disclosure

The authors report no conflicts of interest in this work.

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