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HCC and angiogenesis: possible targets and future directions

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Abstract

Hepatocellular carcinoma (HCC), the most common primary liver tumor, is notoriously resistant to systemic therapies, and often recurs even after aggressive local therapies. HCCs rely on the formation of new blood vessels for growth, and VEGF is critical in this process. A hallmark of new vessel formation in tumors is their structural and functional abnormality. This leads to an abnormal tumor microenvironment characterized by low oxygen tension. The liver is perfused by both arterial and venous blood and the resulting abnormal microenvironment selects for more-aggressive malignancies. Anti-VEGF therapy with sorafenib was the first systemic therapy to demonstrate improved survival in patients with advanced-stage HCC. This important development in the treatment of HCC raises hope as well as critical questions on the future development of targeted agents including other antiangiogenic agents, which hold promise to further increase survival in this aggressive disease.

Introduction

Despite many treatment options for patients with early-stage hepatocellular carcinoma (HCC), the mortality rate remains high making HCC the third leading cause of cancer-related death worldwide.[1] This high mortality rate reflects the poor prognosis for patients with advanced-stage HCC, the pattern of presentation, and the poor outcome associated with cirrhosis. Most patients present with advanced-stage disease, only 30% of patients present with resectable disease, and up to 80% have underlying cirrhosis.[2] The treatment options in advanced-stage disease are limited, and the survival rate is dismal. Thus, novel therapeutic approaches are desperately needed.

Primary tumors of the liver can be classified as either benign or malignant and by the cell type of origin (mesenchymal or epithelial). HCC is the most frequently occurring type, accounting for 90% of all primary malignant liver cancers, but others include intrahepatic cholangiocarcinoma, mixed HCC and cholangiocarcinoma, angiosarcoma, hepatoblastoma, and epithelioid hemangioendothelioma.[3] The growth of a liver tumor requires the formation of new blood vessels, which has provided a strong rationale for antiangiogenic strategies as

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Competing interests
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therapy.[4,5] Indeed, antiangiogenic agents that inhibit the VEGF pathway have been approved for cancer treatment (for example, sorafenib for advanced-stage HCC[4] or bevacizumab in combination with chemotherapy for metastatic colorectal cancer[7]). Unfortunately, less than half of patients with advanced-stage HCC benefit from these therapies, and the benefits are transient.[6] Finally, aggressive anti-vascular therapies are available for unresectable HCC—hepatic artery ligation (HAL) and transcatheter arterial chemoembolization (TACE). Unfortunately, aggressive tumor regrowth typically occurs, likely due to exacerbation of tumor hypoxia, surge in VEGF expression, and inflammation.[8] However, judicious administration of anti-VEGF or anti-placental growth factor (PlGF) treatments can transiently 'normalize' the tumor vasculature,[5,8] which could potentially enhance the efficacy of radiation and chemotherapy by alleviating hypoxia and tumor invasiveness.[9,10]

Two key challenges have hampered progress. First, modeling HCC in mice has been difficult. *Ex vivo* and subcutaneous *in vivo* models provide critical cell biology and response data, but do not capture the important interactions occurring between HCC cells and the inflammatory local and 'distant' (bone marrow-derived) stroma. Most models do not have underlying cirrhosis—a condition that occurs in 80% of human HCC. Given the critical role that inflammation has in the initiation of HCC—in particular interleukin (IL)-6[11]—establishing novel models that capture the characteristics of human disease will be key for testing future therapies. Second, response assessment has been a challenge. Therapy-induced necrosis or vascular normalization may not lead to tumor shrinkage in HCC and can mask the therapeutic effects of antiangiogenic agents.[12,13] Thus, establishing techniques that can measure and/or predict the antitumor effects of antiangiogenics will be critical for testing future therapeutic strategies.

We discuss the current understanding of new blood vessel formation in HCC, and review the cellular and molecular mechanisms involved, the insights that emerged from preclinical and clinical studies of antiangiogenic therapies, and the potential strategies and biomarkers for optimally developing novel antiangiogenic therapies.

Angiogenesis in HCC

Normal liver is organized in lobules segregated by interlobular connective tissue and containing 'cords' of hepatic parenchymal cells and hepatocytes, which surround a central vein and are separated by vascular sinusoids. Sinusoidal liver endothelium is fenestrated and lacks a basement membrane. The fenestrations permit blood plasma to surround the exposed surfaces of the hepatocytes through the space between the fenestrated endothelium and the cells—the space of Disse—which contains collagen fibers and fibroblasts. Liver perivascular cells (pericytes) are the hepatic stellate cells localized in the space of Disse. The stellate cells have a major role in liver fibrosis—the formation of scar tissue in response to liver damage. Kupffer cells (liver macrophages that take up and destroy the pathogens that enter the blood in the intestine) are also closely associated with the sinusoids. Blood from the portal vein and hepatic artery mixes together in the hepatic sinusoids, and after 'filtration' by hepatocytes drains out of the lobule through the central hepatic vein.

Liver tumors display marked vascular abnormalities. Aberrant microvasculature typically may seem 'arterialized' (tight vessels covered by smooth muscle cells) and/or 'capillarized' (capillaries without fenestration and with laminin basement membrane deposition),[14] and is less dense than normal liver vasculature.[15] Liver tumor vessels have an abnormal blood flow and are excessively leaky. In turn, this leads to hypovascular areas and severe hypoxia and/or necrosis—all hallmarks of liver tumors. Although HCC is a highly angiogenic cancer, it is characterized by hypoxia. Hypoxia may promote HCC growth and progression and

resistance to therapies.[16] Conversely, inducing vessel normalization and alleviating hypoxia delays HCC growth.[5]

Overexpression of VEGF leads to focal leaks in tumor vessels, causing nonuniform blood flow and heterogeneous delivery of drugs and oxygen.[17] VEGF is largely responsible for abnormal structure and function of liver tumor vessels. In addition, VEGF can function as a cytokine and may directly affect the hepatic stellate cells, the Kupffer cells, hepatocytes or the cancer cells themselves if they depend on VEGF receptors for their survival or function.[18,19] VEGF expression can be independently regulated by hypoxia and acidosis.[20] VEGF expression is regulated by oncogenic gene mutations, hormones, cytokines and various signaling molecules (nitric oxide, MAP kinases).[21–23] Moreover, VEGF may be released by stromal cells and from the extracellular matrix, the latter via matrix metalloproteinase (MMP)-9-mediated proteolysis.[24,25] High VEGF expression is often seen in chronic liver disease.[26]

Solid tumors use different mechanisms such as sprouting, intussusception or co-option of local vasculature or incorporation of circulating vascular precursors to acquire new blood vessels (Figure 1).[21] Owing to the heterogeneity of tumor endothelial cell phenotypes in HCC and the clear distinction between endothelial cells from the normal and malignant liver, it is conceivable that both local and circulating cells contribute to new vessel formation.[8,27] Unfortunately, studying these mechanisms in liver cancer is a major challenge. First, preclinical models often fail to reproduce all features of human disease. Second, tumors have already induced new vessel formation at the time of diagnosis and/or surgery.

The molecular pathways involved in liver tumor angiogenesis are incompletely characterized. Currently, the main targets for the antiangiogenic agents in development for liver cancer therapy are VEGF and its receptors VEGFR1 and VEGFR2. However, an increasing number of molecular pathways involved in blood vessel formation have been identified. We discuss the key proangiogenic growth factors and inflammatory molecules identified in liver tumors (Boxes 1 and 2 and Supplementary Table 1 online).

Box 1

Molecular mechanisms of angiogenesis in liver

The effects of VEGF are primarily mediated via VEGFR2 in endothelial cells.[21,22,113] Tumor vessels dilate and become leaky in response to VEGF. MMPs, Ang2 and VEGF mediate the dissolution of the vascular basement membrane and the interstitial matrix. A variety of molecules promote endothelial proliferation, migration and assembly into vascular networks, including VEGF, Ang1, Ang2 and bFGF.[21] Endothelial cell migration and spreading in response to growth factor signaling is mediated by $\alpha_v\beta_5$, $\alpha_v\beta_3$, and $\alpha_5\beta_1$ integrins.[114] Quiescent endothelial cells may survive for several years in the vessels of normal adult tissues. Soluble receptors for VEGF (VEGFR1 or NRP1[115]) sequester the ligands and reduce angiogenic activity. bFGF is a potent mitogen implicated in angiogenesis, but its role in liver cancer remains to be clarified. Other molecules involved in tumor angiogenesis are PlGF, IGF-I, PAI-1, NOS, COX2, TSP2, PDGF isoforms, and EGF.[21,28,116] The Dll4/Notch pathway is a negative mediator of angiogenesis.[117] Dll4 decreased the expression of VEGFR2 and its co-receptor NRP1.[118,119] An anti-Dll4 antibody decreased endothelial cell proliferation and caused defective cell fate specification or differentiation, and led to tumor growth inhibition in several tumor models.[120] Dll4/Notch1 signaling regulates the number of tip cells that control vessel sprouting and branching by restricting tip-cell formation in response to VEGF.[121] Dll4 might have a role in the progression of liver tumors[122] and may serve as a

potential target. PI3K/Akt that is activated in endothelial cells[123] is being explored as a target for HCC treatment. The levels of angiogenic molecules (VEGF, soluble VEGFR1, PlGF and bFGF) in circulating blood from cancer patients significantly change in response to anti-VEGF treatment.[102]

Abbreviations: Ang, angiopoietin; bFGF, basic fibroblast growth factor; COX2, cyclooxygenase-2; Dll4, delta-like protein 4; IGF-I, insulin-like growth factor 1; MMP, matrix metalloproteinase; NOS, nitric oxide synthase; NRP, neuropilin; PAI-1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; PIGF, placental growth factor; TSP, thrombospondin.

Box 2

Inflammatory molecules and their potential role in liver cancer angiogenesis

Chronic inflammation is a potential precursor of liver carcinogenesis.[11,124] In liver cancer, NFκB is involved in tumor initiation and progression mediated via STAT3 activation.[125–127] Inflammatory cytokines induced by NFκB might affect angiogenesis directly via endothelial cells, or indirectly by cancer cells or recruitment and/or activation of inflammatory cells.[8,128] IL-1α has a critical role[129] by recruitment of inflammatory cells.[130] TNF-α can also promote tumor progression by different pathways: direct effect on tumor cells, induction of CXCR4 and stimulation of epithelial–mesenchymal transition.[131] TNF-α promotes cell survival and angiogenesis or induces endothelial cell apoptosis, and vascular disruption and increased permeability. IL-6 is induced by NFκB and other transcription factors (C/EPBb and AP-1), and modulates inflammation via IL-6R and gp130. Smooth muscle cells, T cells and macrophages secrete IL-6 to stimulate immune responses and promote inflammation. IL-6 may also have anti-inflammatory effects by inhibition of TNF-α and IL-1, and activation of IL-1Ra and IL-10. The proliferative and survival effects of IL-6 are mediated by STAT3.[11] In HCC, IL-8 may have a role in cell invasion.[132,133] IL-8 can promote tumorigenesis and angiogenesis through CXCR1 and CXCR2, and the Duffy antigen receptor for cytokines, which has no defined intracellular signaling capabilities.[134] Overexpression of VEGF induces SDF1α expression, and SDF1α and CXCR4[135] may drive cell migration and angiogenesis by VEGF-independent mechanisms.[136] SCF is the ligand for c-KIT, primarily expressed by early hematopoietic precursors. While c-KIT expression is rarely detectable in HCC, both SCF and c-KIT are expressed during cholangiocarcinogenesis.[137]

Abbreviations: AP-1, activator protein 1; C/EPB, CAAT/enhancer binding-protein; CXCR, CXC-chemokine receptor; HCC, hepatocellular carcinoma; IL, interleukin; NFκB, nuclear factor κB; SCF, stem cell factor; SDF1α, stromal-cell-derived factor 1α; STAT, signal transducers and activators of transcription; TNF, tumor necrosis factor.

Angiogenesis and clinical outcomes

Angiogenesis is initiated by destabilization of existing microvasculature, which leads to vascular hyper-permeability, remodeling of the extracellular matrix, and endothelial cell activation. Upon activation, the endothelial cells proliferate, migrate, and undergo cord formation to form new vessels. Subsequent activation and recruitment of pericytes stabilize the new blood vessels.[22,28,27] During angiogenesis, the expression of proangiogenic factors is balanced by release of antiangiogenic molecules.[30] In HCC, a net excess of angiogenic factors produced by tumor cells, vascular endothelial cells, immune cells and pericytes tips

this balance leading to the activation and recruitment of endothelial cells and pericytes.[4,31] The plasma concentration of proangiogenic growth factors VEGF, angiopoietin-2 (Ang2), and platelet-derived growth factor (PDGF)-B is increased in patients with HCC compared with cirrhotic patients.[32] Other angiogenic factors potentially involved in liver cancer are PlGFs, basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-α, TGF-β, hepatoctye growth factor (HGF), EGF, IL-4, IL-6 and IL-8 (Boxes 2 and 3).[30]

Box 3

Sorafenib in HCC

Sorafenib is an oral multikinase inhibitor that inhibits VEGFR1, VEGFR2, VEGFR3 and PDGFR-α, PDGFR-β, c-KIT, Raf-1 and BRAF. Early evidence of antitumor activity was observed from a phase II study of 137 patients with advanced HCC: TTP was 4.2 months and overall survival 9.2 months.[13] An international phase III trial (SHARP) subsequently demonstrated improved overall survival and TTP. Median survival was 10.7 months in the sorafenib arm versus 7.9 months in the placebo group. Median TTP was 5.5 months in the sorafenib arm versus 2.8 months in the placebo group.[6] The magnitude of this benefit was similar in another phase III study conducted in Asia in patients with advanced-stage HCC. Overall survival was 6.5 months in the sorafenib group versus 4.2 months in the placebo group.[57] The typical response rates for sorafenib in advanced-stage HCC are extremely low (2–3% as evaluated by RECIST). However, tumor necrosis has been reported in those treated with sorafenib, indicating that RECIST may not be an appropriate end point for antiangiogenics in HCC. Toxic effects associated with sorafenib are generally manageable. Grade 3 adverse events included hand–foot skin reactions, diarrhea, and fatigue. No prospective data are available regarding the efficacy and toxicity of sorafenib in patients with HCC with worsening underlying hepatic dysfunction. No validated biomarker is available to predict the clinical benefits from sorafenib. The efficacy of sorafenib in the adjuvant setting or in combination with molecularly targeted agents or chemotherapy remains unknown. Ongoing phase III studies (NCT01004978, NCT00692770, NCT00901901, and NCT01075555) will hopefully provide insight into these critical issues.

Abbreviations: PDGFR, platelet-derived growth factor receptor; HCC, hepatocellular carcinoma; TTP, time to tumor progression

The expression of VEGF and its receptors, which include VEGFR1, VEGFR2, and VEGFR3, is elevated in HCC cell lines and tissues, as well as in the blood circulation in patients with HCC.[32–35] The increase in VEGF expression is seen in cirrhotic and dysplastic liver tissues, suggesting a possible role for VEGF-driven angiogenesis in hepatocarcinogenesis.[36] One study found that VEGF levels were progressively increased through the successive steps of low-grade dysplasia, high-grade dysplasia, and early-stage HCC.[37] In addition, elevated VEGF expression is linked with high HCC tumor grade, vascular invasion, and portal vein invasion.[38–41]

A poor prognosis for patients with HCC is correlated with elevated circulating VEGF levels after surgery, radiofrequency ablation (RFA) or TACE.[42–49] Similarly, high levels of VEGF in HCC tissues correlated with rapid tumor recurrence in patients with HCC.[50–54] There are limited studies on other angiogenic factors as prognostic biomarkers. For example, rapid recurrence after therapy has been linked with higher PlGF, platelet-derived endothelial cell growth factor (PD-ECGF), MMP-2, Ang2 and hypoxia-inducible factor (HIF)-1α levels.[50,51,55,56]

VEGF is a critical player in liver cancer angiogenesis, and its elevation in tumor tissue or in circulation correlates with more-aggressive disease. Thus, future studies should identify and characterize these pathways, with the goal of targeting inherent or acquired resistance to anti-VEGF therapies.

Antiangiogenic therapy of liver cancer

A large number of antiangiogenic agents are currently being tested for the treatment of HCC. We discuss the experience with agents that have reached more advanced phases of development (Table 1).

Sorafenib and sunitinib

Sorafenib, a multi-targeted tyrosine kinase inhibitor (TKI) approved by the FDA for patients with advanced-stage renal cell carcinoma, is the first systemic therapy to improve survival in phase III trials of patients with advanced-stage HCC (Box 3).[6,57] The exact mechanism by which sorafenib benefits patients with advanced-stage HCC remains unknown.

Sorafenib targets VEGF receptors, and is now thought to exert its effect primarily by blocking VEGF signaling, as its efficacy against BRAF is questionable.[58] However, sorafenib has a moderate anti-VEGFR2 activity. Since sorafenib has demonstrated improved overall survival benefits in patients with advanced-stage HCC, its potential value in early-stage disease is being assessed. One such setting is after TACE, to counteract the surge in VEGF,[46,47] and sorafenib is being tested either concurrently or after TACE in clinical trials.[59] An ongoing randomized phase III trial of adjuvant sorafenib will test if this agent reduces the high recurrence rates of HCC after surgical resection. However, it should be noted that anti-VEGF therapy has failed to show benefit in the adjuvant setting in colorectal cancer, despite its efficacy in metastatic disease.[60,61]

Sunitinib is an oral multi-targeted TKI with more potent activity against VEGFR1 and VEGFR2 compared with sorafenib. It also targets PDGFR-α, PDGFR-β, c-KIT, FLT3, RET and other kinases.[62–64] Currently, clinical data of sunitinib efficacy in HCC are based on four single-arm phase II studies that used three different dose schedules (Supplementary Table 2 online).[12,65–67] Three of the studies used the standard 4-weeks-on, 2-weeks-off regimen (6 weeks per cycle), which was efficacious in patients with renal cell carcinoma and gastrointestinal stromal tumors.[68,69] The studies showed activity for sunitinib in advanced-stage HCC, but indicated that the higher 50 mg dose may not be well tolerated in this patient population.[12,65,66] Koeberle and colleagues reported that continuous 37.5 mg daily dosing has comparable safety and efficacy profiles to the intermittent regimens (Supplementary Table 2 online).[67] To date, no randomized study has compared directly the intermittent versus continuous schedule for efficacy and tolerability. Nevertheless, a randomized phase III study comparing sunitinib with sorafenib in advanced-stage HCC (SUN 1170) used the continuous daily dosing of 37.5 mg of sunitinib. This was based on preclinical results and anecdotal clinical evidence that intermittent regimens may promote tumor progression during treatment breaks.[70] While these observations await confirmation in controlled clinical trials, the SUN 1170 trial was stopped early because of a higher incidence of serious adverse events in the sunitinib arm, and because sunitinib did not demonstrate superiority or non-inferiority to sorafenib. Since the full dataset from this trial are not available, it remains unknown if the toxicity associated with this dose schedule and study design contributed to the failure of sunitinib in this study. However, further development of sunitinib in HCC is unlikely. This failure raises important questions regarding the mechanism of action and predictive biomarkers for antiangiogenic agents in this tumor type. Answering these questions will be critical for the development of other anti-VEGF agents.

Specific or selective VEGFR blockers

Ramucirumab (IMC-1121B, ImClone Systems and Eli Lilly, NJ, USA) is a recombinant human monoclonal antibody that binds to the extracellular domain of VEGFR2. Intravenous ramucirumab given biweekly at a dose of 8 mg/kg in patients with advanced-stage HCC showed a median progression-free survival (PFS) of 4.0 months and median overall survival of 12 months with limited toxic effects in a single-arm phase II study.[71] A phase III study of best supportive care plus ramucirumab or placebo in patients with advanced-stage HCC who failed to respond to sorafenib (REACH trial) is planned (Table 1).

Bevacizumab is a recombinant, humanized mono-clonal antibody that targets VEGF, and is approved by the FDA for the treatment of advanced-stage colorectal, lung, breast, renal and brain cancers. In addition to its direct antiangiogenic effects, bevacizumab may enhance chemotherapy administration by 'normalizing' tumor vasculature and decreasing the elevated interstitial pressure in tumors.[9,10,72,73] Several studies have explored the use of bevacizumab either as a single agent or in combination with cytotoxic or molecular-targeted agents in patients with advanced-stage HCC (Supplementary Table 3 online).[74–79] As a single agent, bevacizumab administered intravenously once every 2 weeks at 5 mg/kg or 10 mg/kg produced a median PFS of 6.9 months and median overall survival of 12.4 months in patients with HCC.[74] Bevacizumab combined with gemcitabine and oxaliplatin (GEMOX-B) produced a median PFS of 5.3 months and overall survival of 9.6 months in advanced-stage HCC.[75] Bevacizumab and erlotinib produced a median PFS of 9 months and overall survival of 15 months in patients with advanced-stage HCC.[79] Despite the early evidence of activity, no registration study is currently planned for bevacizumab in patients with HCC.

Linifanib (ABT-869, Abbott Laboratories, IL, USA) is a TKI that has potent activity against VEGFR and PDGFR.[80] Preliminary data from an open-label, multicenter phase II study of linifanib given at 0.25 mg/kg daily in patients with advanced-stage HCC showed a median time to tumor progression (TTP) of 3.7 months and overall survival of 9.7 months, with a tolerable safety profile.[81] This finding has encouraged further development of linifanib in HCC, and a phase III study comparing linifanib with sorafenib is ongoing (Table 1).

Cediranib (AZD2171, AstraZeneca, Cheshire, UK) is an oral pan-VEGFR TKI with activity against PDGFR and c-KIT. Cediranib is a potent inhibitor of both VEGFR2 and VEGFR1.[82] A small phase II trial of daily cediranib at a dose of 45 mg showed a high rate of grade 3 adverse effects (primarily fatigue), which frequently lead to treatment discontinuation.[83] Another phase II study of cediranib at 30 mg daily in patients with HCC conducted at our institution is ongoing, and the results are pending (Table 1).

Pazopanib (GW786034, GlaxoSmithKline, Brentford, UK) is an oral TKI that targets VEGFRs, PDGFRs, and c-KIT, and was recently approved by the FDA for advanced-stage renal cell carcinoma. A phase I study determined the maximum tolerated dose (MTD) of 600 mg once daily for pazopanib in advanced-stage HCC. The median TTP was 137.5 days.[84]

Vatalanib (PTK787, Novartis, Basel, Switzerland) is an oral TKI which targets VEGFRs, PDGFRs, and c-KIT.[85,86] A phase I study of single-agent vatalanib given at 750 mg or 1,250 mg per day induced stable disease in nine of 18 patients with unresectable HCC who were evaluable for response.[87] A phase I–II study of daily vatalanib with doxorubicin (60 mg/m^2 every 3 weeks) in advanced-stage HCC showed that patients treated at the MTD for vatalanib had a median PFS of 5.4 months and overall survival of 7.3 months.[88] Vatalanib development has been discontinued due to an industry decision.

Dual blockers of VEGF and bFGF pathways

Brivanib alaninate (Bristol-Myers Squibb, NJ, USA) and TSU-68 (SU6668, Taiho Pharmaceutical, Tokyo, Japan) are dual inhibitors of VEGF and FGF receptors. Preclinical reports showed that brivanib treatment can inhibit HCC growth and that TSU-68 can normalize tumor vasculature in mouse xenograft models.[89,90] A phase II study was conducted to assess the efficacy and safety of daily brivanib (800 mg) in patients with advanced-stage HCC. In patients who had received no prior systemic therapy, a median overall survival of 10 months, TTP of 2.8 months and manageable adverse effects were reported.[91] A phase I–II trial of TSU-68 in heavily pretreated patients with advanced-stage HCC established the MTD at 200 mg twice daily and showed a median TTP of 2.1 months and survival of 13.1 months.[92] Currently, brivanib is being evaluated in phase III studies in the first-line setting versus sorafenib, and in the second-line setting in patients with sorafenib-refractory advanced-stage HCC (Table 1).

Multitargeted inhibitors of VEGFR

Vandetanib (ZD6474, AstraZeneca, Cheshire, UK) is a TKI with activity against VEGFR2, EGFR and RET. A randomized phase II study of vandetanib in advanced-stage HCC is ongoing (Table 1). Foretinib (GSK1363089, XL-880, GlaxoSmithKline, Brentford, UK) is an oral TKI that selectively inhibits c-Met and VEGFR2. A phase I study of foretinib has established the MTD at 240 mg, given on the first 5 days of a 14-day cycle.[93] A phase I–II study of foretinib in advanced-stage HCC is ongoing (Table 1).

Toxic effects of antiangiogenic therapy

With the increasing use of antiangiogenic therapy, certain ‘class’ toxicity profiles have emerged, which include hypertension, bleeding, thromboembolic events and proteinuria. Other toxic effects are more specific for TKIs, such as hand–foot skin reaction and rash. Whether any of these adverse effects are associated with clinical outcome remains to be determined in future trials.

Biomarkers: progress and challenges

Antiangiogenic therapies have brought new promise for HCC therapy, but have also changed the needs and expectations of how imaging modalities can be used to determine the efficacy of these treatments. This is because the mechanisms of action of these new agents are inconsistent with the assessment of response by RECIST.[94–96] For example, if these therapies cause tumor necrosis this effect may induce no shrinkage or even an apparent enlargement of the tumor due to cystic changes and edema.[97] Therefore, the European Association for the Study of the Liver (EASL) guidelines recommended that assessment of tumor response should incorporate the reduction in viable tumor burden.[98] However, whether the current imaging techniques allow consistent quantification of tumor necrosis and if this is a meaningful end point after antiangiogenic therapy in HCC remains unclear.

The structural and functional abnormalities of tumor vessels may be reversed by antiangiogenic therapies.[9] Detecting these responses requires functional, ‘vascular’ imaging. Functional imaging has conventionally been the domain of nuclear medicine. However, the high spatial resolution, easy availability and technologic innovations in imaging have opened the doors for establishing techniques such as dynamic contrast-enhanced (DCE) MRI, perfusion CT, and DCE ultrasonography to evaluate treatment response. On contrast-enhanced CT and MRI, tumor enhancement characteristics are influenced by several parameters such as blood flow, blood volume fraction, blood vessel permeability and distribution volume fraction. However, the tumor physiologic features can be quantified by applying appropriate mathematic modeling (Box 4).[99]

Box 4

Functional imaging of tumor vasculature in hepatocellular carcinoma

Perfusion CT (CTp) is being increasingly used for quantification of tumor vascular density and angiogenesis, and may permit evaluation of tumor response to antiangiogenic agents.[138–140] In advanced disease, CTp after bevacizumab has shown significant decreases in tumor blood flow, blood volume, and permeability-surface area and an increase in mean transit time (MTT). Moreover, baseline MTT values and the change after bevacizumab correlated with a better clinical outcome.[141] These changes are tumor specific as the HCCs exhibit substantial changes in their perfusion parameters such as K^{trans} and blood volume after bevacizumab treatment without any significant changes in these parameters in vessels of the caudate lobe and spleen.[142] Similarly, the contrast-enhancement patterns in HCC obtained by dynamic contrast-enhanced (DCE) MRI are influenced by tumor angiogenesis and correlate with tumor microvascular density and VEGF expression.[143] Thus, suppression of tumor vascular permeability induced by antiangiogenic agents can be reliably detected and quantified by DCE MRI. For example, sunitinib treatment in patients with advanced-stage HCC led to rapid and significant decreases in K^{trans}.[12] The extent of decrease in K^{trans} was substantially higher in patients who experienced partial response or stable disease compared with that in patients with progressive disease or who died during the first two cycles of therapy.[12] This is consistent with the effects of antiangiogenic agents in recurrent glioblastoma and the potential predictive biomarker value of the rapid decrease in K^{trans}.[144,145] Data are emerging to support DCE ultrasonography (DCE US) as a valuable and less expensive second level imaging modality.[146] Quantitative functional evaluation by DCE US performed at day 3 and 14 was able to predict response at 2 months in patients with HCC treated by bevacizumab.[147]

Despite this progress, important challenges remain with the use of these imaging biomarkers. First, there is no consensus on how to use CT to assess response to antiangiogenic therapies in liver tumors.[100] Estimates of viable tumor volume or extent of tumor necrosis in HCC to predict the outcome of patients after antiangiogenic treatment are promising.[101] The process of estimation of tumor volume-—although feasible on all commercially available image-processing workstations—is not fully automated and demands expertise and dedicated personnel. Therefore, it is not currently integrated into routine oncologic imaging workflow. The novel antiangiogenic agents currently in clinical development vary in their ability to induce tumor necrosis, which adds to the complexity of obtaining total liver tumor volume as a surrogate end point.[94] Likewise, imaging of tumor angiogenesis and vascular responses to antiangiogenic therapies will require routine availability of state-of-the-art dynamic imaging technologies and local expertise, robust and reliable analysis of results and a mandatory customization of imaging protocols in clinical trials. Finally, due to the inherent complexity of these novel imaging modalities and high costs, it remains a challenge how to integrate these methods in large phase III studies to prospectively validate some of these potentially useful imaging end points and biomarkers.

Future directions

Future research needs to improve our understanding of antiangiogenic therapy for HCC. While most pharmaceutical companies are developing selective or potent anti-VEGF agents, it is likely that progress will come from the use of agents targeting multiple proangiogenic factors (for example, bFGF, c-Met, Ang2, PlGF, stromal-cell-derived factor 1α [SDF1α]). The paucity of data from preclinical models limits our understanding of the relevance of these targets in HCC. Nevertheless, several trials with agents targeting VEGF and FGFR or

c-Met are underway. Novel strategies combining antiangiogenic agents with chemotherapy or other molecular-targeted agents are urgently needed. However, neither sorafenib nor any of the other anti-VEGFR TKIs under development in HCC has shown an increase in survival when combined with chemotherapy. Predictive biomarkers are urgently needed for antiangiogenic therapy.[102] Circulating biomarkers show promise in identifying patients most likely to benefit from antiangiogenic therapies: changes in α-fetoprotein (AFP), IL-6, SDF1α, soluble c-KIT, soluble VEGFR1, VEGF-C, IL-8, TNF-α, Ang2, soluble VEGFR2, collagen IV and in circulating monocytes and circulating progenitor cells have been shown in exploratory studies to associate with outcome of treatment in HCC (Table 2). These biomarker candidates need to be validated in large prospective studies.

The critical importance of biomarker discovery and validation for antiangiogenic agents in advanced-stage HCC is exemplified by the following: first, our poor understanding of the mechanism by which sorafenib benefits patients; second, the recent failure of sunitinib; third, the largely equivalent and modest efficacy observed in all phase II trials of other anti-VEGF agents conducted to date; and finally, the serious toxic effects and the high costs of these therapies. Unfortunately, the limited resources continue to be a challenge for conducting clinical trials incorporating biomarker studies in HCC.

There is an urgent need to identify ‘druggable’ primary and acquired resistance and/or escape pathways in relevant preclinical models of HCC, in order to guide the design of improved treatment strategies. HCC etiology is inextricably linked to inflammation, as a result of focal hypoxia and necrosis inside these tumors and by enhanced expression of VEGF and other cytokines.[103] Cytokines may be important in recruiting circulating progenitor cells to tumor tissue.[104] Indeed, VEGF blockade by sunitinib affected both the tumor vasculature and the ‘distant stroma’, that is, bone marrow-derived progenitor cells and their progeny in advanced HCC (Figure 1).[12,105]

The time-dependent changes in the number of circulating progenitor cells in the blood, and the plasma concentration of IL-6 and SDF1α after sunitinib significantly correlated with outcome.[12] Circulating progenitor cells were considerably decreased by sunitinib, probably due to additional inhibition of c-KIT and FLT3 in hematopoietic progenitor or stem cells.[106] Hematologic toxic effects are frequent side effects of anti-VEGF agents. Indeed, sunitinib significantly and rapidly decreased all myeloid and lymphoid circulating cell populations.[106] The extent of the early decrease in neutrophils, platelets and monocytes, as well as the development of nonhematologic toxic effects (skin toxicities), was significantly associated with improved survival outcomes.[106] These observations suggest that the effects of these types of agents on the hematopoietic system are rapid, may be directly related to their activity in advanced-stage HCC, and could potentially be used to predict survival outcomes in advanced-stage HCC. This paradigm has been proposed for other toxic effects such as hypertension or skin toxicity, and deserves further investigation given the role of inflammation in liver cancer. In particular, mechanistic preclinical and clinical studies should determine how this information could be used therapeutically. For example, should anti-VEGF therapy be combined with anti-inflammatory agents or anti-SDF1α or anti-CXCR4 agents to go beyond what is achievable with anti-VEGF agents alone?

Conclusions

Approval of sorafenib for HCC has opened a new era for antiangiogenic therapies in this disease, which is notoriously resistant to systemic therapies. However, the initial enthusiasm has been tempered by recent failures or modest efficacy of other antiangiogenic agents. This underscores the need for thorough, mechanistic investigations in relevant preclinical models and well-designed, randomized studies of this highly heterogeneous disease. These

approaches should lead to a better selection of patients for antiangiogenic therapy based on biomarkers, and should provide critical insight into the mechanisms of resistance, thus facilitating the discovery of new targets. In turn, this may finally allow us optimize the current therapies for this dreadful disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

- Hepatocellular carcinoma (HCC) is a heterogeneous disease with multiple etiologies that is uniformly fatal when unresectable; other malignant liver tumors include cholangiocarcinoma, angiosarcoma, hemangioendothelioma and hepatoblastoma
- The growth of HCCs depends on their ability to recruit blood vessels by forming new vessels through sprouting (angiogenesis) and potentially by recruiting proangiogenic bone marrow-derived cells
- Tumor neovasculature is highly abnormal, both structurally and functionally because of overexpression of VEGF; other molecules are also involved and may be important therapeutic targets
- Sorafenib was developed as a VEGFR2, VEGFR3, PDGFR-β, and Raf/MEK/ERK signaling inhibitor; despite being standard of care in advanced-stage HCC, its mechanism of action remains unknown
- Antiangiogeneic agents can transiently prune and normalize the tumor vasculature and improve the outcome of other treatments (chemotherapy, radiation) given during the normalization window
- Circulating and imaging markers may be useful as pharmacodynamic end points, and may hold promise as potential surrogate and predictive markers for antiangiogenic therapy

Review criteria

Information on antiangiogenesis in hepatocellular carcinoma (available from the NIH databases) and the publications related to clinical studies were retrieved from the NIH website (www.clinicaltrials.gov). PubMed was searched for studies of angiogenesis and antiangiogenic agents published before 6 January 2011, including early-release publications. Search terms included “hepatocellular carcinoma”, “clinical trial”, “biomarker”, “anti-angiogenesis”, “anti-vascular”, “imaging”, and “tyrosine kinase inhibitor”. Full articles were checked for additional material when appropriate. Data published in abstract form from ASCO meetings from 2006 to 2010 and the 2009 Radiological Society of North America (RSNA) meeting were also included.

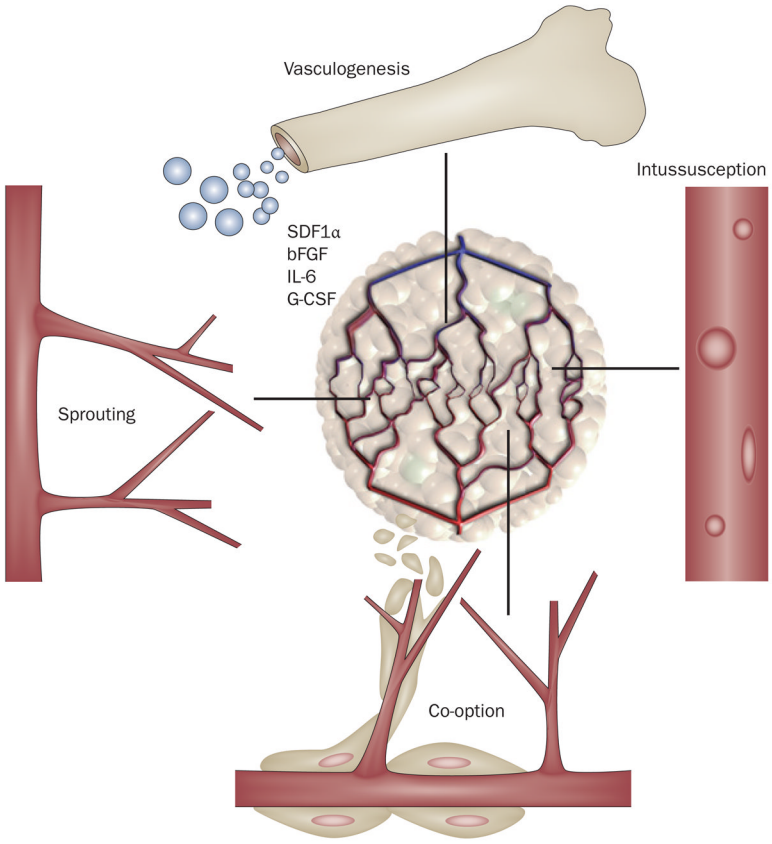

Figure 1.
Schematic representation of potential escape mechanisms from anti-VEGF therapy. HCCs might use four potential mechanisms to acquire new blood vessels for their growth and after VEGF blockade: co-option, angiogenesis (sprouting), vasculogenesis (bone-marrow-derived endothelial progenitor cell recruitment to increase the tumor vascular supply) and intussusception. SDF1α, bFGF, IL-6 and G-CSF are increased in the circulation of patients with HCC treated with anti-VEGF agents. These molecules may potentially contribute to HCC neovascularization during VEGF-pathway inhibition. Permission obtained from Nature Publishing Group © Carmeliet, P. & Jain, R. K. *Nature* **407**, 249–257 (2000).
Abbreviations: bFGF, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; HCC, hepatocellular carcinoma; IL, interleukin; SDF1α, stromal-cell-derived factor 1α.

Table 1

Antiangiogenic agents in development for HCC*

Agent and manufacturer	Drug targets	Stage of development (NCI trial identifier)
Sorafenib (Nexavar, Bayer and Onyx)[7,57]	Oral multikinase inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFR-α, PDGFR-β, Raf-1, p38MAPK, Flt-3, c-KIT, RET	Approved for the treatment of HCC
Brivanib (BMS-582664, Bristol-Myers Squibb)[91]	Oral TKI against VEGFR2 and FGFR-1	Phase II–III (NCT00858871, NCT00825955, NCT01108705)
Linifanib (ABT-869, Abbot)[107]	Oral selective TKI against VEGFR, PDGFR	Phase II–III (NCT01009593)
Pazopanib (GW786034)[84]	Oral TKI targeting VEGFR, PDGFR, and c-KIT	Phase I
Vatalanib‡ (PTK787, Novartis)[87,88]	Oral tyrosine kinase inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFR, c-KIT	Phase I–II
Cediranib (AZD2171, Recentin, AstraZeneca)[83]	Oral tyrosine kinase inhibitor of VEGFR1, VEGFR2, VEGFR3	Phase II (NCT00427973)
Ramucirumab (IMC-1121B, ImClone Systems and Eli Lilly)[71]	Recombinant human monoclonal antibody that binds to the extracellular domain of the VEGFR2	Phase II–III (NCT00627042)
TSU-68 (SU6668, Taiho)[92]	Oral angiogenesis inhibitor targeting VEGFR, PDGFR, and FGFR	Phase I–II (NCT00784290)
Vandetanib (ZD6474, Zactima, AstraZeneca)	Oral dual inhibitor of VEGFR and EGFR	Phase II (NCT00508001)
Foretinib (GSK1363089; XL-880, GlaxoSmithKline)	Oral dual inhibitor of VEGFR and c-Met	Phase II (NCT00920192)

*See Supplementary Tables 2 and 3 online for sunitinib and bevacizumab studies.

‡Discontinued. Abbreviations: FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; NCI, National Cancer Institute; PDGFR, platelet-derived growth factor receptor; TKI, tyrosine kinase inhibitor.

Table 2

Blood-based biomarkers for antiangiogenic therapy in advanced HCC

Biomarker candidate	Agent	Correlation with outcome
AFP[12,108]	Sorafenib, bevacizumab, sunitinib	Greater decrease in serum AFP; better PFS and OS
IL-6[12]	Sunitinib	Greater increase correlates with lower survival
SDF1α[12]	Sunitinib	Greater increase correlates with lower survival
Soluble c-KIT[12]	Sunitinib	Greater decrease on treatment improved PFS and OS
Soluble VEGFR1[12]	Sunitinib	Elevated levels at progression
VEGF-C[109]	Sunitinib	Elevated levels in responders
IL-8[12,110]	Sunitinib	Elevated levels correlate with a shorter PFS and OS
TNF-α[12]	Sunitinib	Elevated levels correlate with a shorter OS
Ang2[111]	Bevacizumab	Higher Ang2 and decreased OS
Soluble VEGFR2[111]	Bevacizumab	Higher sVEGFR2 and decreased PFS
Collagen IV[91]	Brivanib	Greater decrease in collagen IV correlates with PFS and OS
Circulating monocytes[105]	Sunitinib	Greater decrease; longer PFS and OS
Circulating progenitor cells[12,112]	Sunitinib	High values on treatment correlate with a low OS; high baseline correlates with a low OS and PFS

Abbreviations: AFP; α-fetoprotein; Ang2, angiopoietin-2; HCC, hepatocellular carcinoma; IL, interleukin; OS, overall survival; PFS, progression-free survival; SDF1α, stromal-cell-derived factor 1α; TNF, tumor necrosis factor.