Molecular targeting for treatment of advanced hepatocellular carcinoma

Il Han Song, M.D. Ph.D.

Department of Internal Medicine, Dankook University College of Medicine, Cheonan, Korea

Hepatocellular carcinoma (HCC) is a major global health problem, which has a grave morbidity and mortality. Over the past few decades, no effective systemic therapeutic modalities have been established for patients with the unresectable HCC in advanced stage. Sorafenib is a small molecule that blocks cancer cell proliferation by targeting the intracellular signaling pathway at the level of Raf-1 and B-Raf serine–threonine kinases, and exerts an anti-angiogenic effect by targeting the vascular endothelial growth factor receptor-1, 2 and 3, and platelet-derived growth factor receptor-β tyrosine kinases. Recently, two clinical successful applications, SHARP and Asia-Pacific trial, of multikinase inhibitor sorafenib represent a significant advance in the treatment of advanced HCC patients without a curative chance. However, because the results of clinical trials show diverse responses in a subset of HCC patients, a molecular classification of HCC through the excavation of specific biomarkers related to its biological behavior is necessary for sorting HCC patients to each group with a biological homogeneity, ultimately leading to the most suitable individualization of molecular targeted therapy in HCC. (Korean J Hepatol 2009;15:299-308)

Key words: Hepatocellular carcinoma; Molecular targeted therapy; Sorafenib; Hepatocarcinogenesis; Signaling pathway

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Abbreviations: BCLC, Barcelona Clinic Liver Cancer; dsRNA, double stranded RNA; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; EMEA, European Medicines Agency; ERK, extracellular signal-regulated kinase; FDA, Food and Drug Administration; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIF, hypoxia-inducible factor; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; MET, mesenchymal-epithelial transition factor; mTOR, mammalian target of rapamycin; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PTK, phosphoinositide 3-kinase; RECIST, Response Evaluation Criteria in Solid Tumors; RNAi, RNA interference; SHARP, Sorafenib Hepatocellular Carcinoma Assessment Randomised Protocol; shRNA, short hairpin RNA; siRNA, small interfering RNA; TACE, transcatheter arterial chemoembolization; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TTP, time to progression; VEGFR, vascular endothelial growth factor receptor

Corresponding author: Il Han Song, E-mail: ilhsong21@dankook.ac.kr; Phone: (041) 550-3924; Fax: (041) 556-3256

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Introduction

Hepatocellular carcinoma (HCC), a major world health problem, is the fifth most common malignancy, affecting over half a million persons per year worldwide and ranking as the third leading cause of cancer-related mortality following only lung and stomach cancers. In general, the geographic prevalence of HCC occurrence is based on the epidemiologic distribution and natural history of hepatitis B virus (HBV) infection, particularly the endemcity of Asia and Africa for chronic HBV infection, although the incidence of HCC has been reported to be increasing in the United States and Europe related to increased prevalence of hepatitis C virus (HCV) infection over recent decades.

HCC in its early stages is a potentially curable disease, but only 30~40% of all HCC patients are eligible for curative treatments, such as surgical resection, liver transplantation or loco-regional ablation at the time of diagnosis, and the remaining majority of patients are subjects for non-curative treatments, such as transcatheter arterial chemoembolization (TACE), systemic chemotherapy, radiotherapy or conservative management of symptoms. There are several factors that may explain this therapeutic limit to a cure in patients with HCC. First, HCC is usually prone to tumor progression, both regional invasion and distant metastasis, due to the aggressive biologic behavior of HCC itself. Second, most cases of HCC are accompanied by chronic liver diseases such as cirrhosis that can interfere with the curative management of HCC. Finally, many cases of HCC are diagnosed in the advanced stage, which means a lack of effective treatment options for enhancing the curative chance.

Recently, sorafenib, a potent multikinase inhibitor, which has been approved for the treatment of patients with unresectable HCC by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMEA), was formally introduced to advanced HCC patients as a molecular targeted therapy. The advance of this therapeutic molecule for targeting the pathogenetic signals relevant to HCC development or progression now represents a break-through in clinical management of this advanced malignancy without other effective therapeutic options. The present review will summarize the potential molecular therapeutic targets in HCC, through which the hepatocarcinogenesis via cell membrane receptors and intracellular signal transduction pathways is mediated, discuss the clinical applications of this kind of targeted therapy which have been published previously and are ongoing under clinical investigations, and consider further therapeutic perspectives for the clinical setting.

Molecular targets and relevant therapeutic molecules

The lack of clinical efficacy of existing systemic chemotherapies and subsequent dismal prognosis in unresectable HCC patients with macrovascular invasion or extrahepatic metastasis have prompted many biologic and clinical researchers to identify the molecular mechanisms implicated in hepatocarcinogenesis. The molecular pathogenesis of HCC is complex and heterogeneous, comprising a multistep process that accounts for the serial accumulation of genomic alterations involved in the development and progression of the tumor. Further understanding of pathogenetic characterization of
HCC at the molecular level, which mainly consists of the specific interaction of both the extracellular growth factors–receptors and the intracellular signal transduction pathways, also helps researchers identify the potential therapeutic targets in a certain step of signaling cascades for the treatment of this tumor. Table 1 displays the molecular targets, their cellular functions, and relevant therapeutic compounds that are currently being evaluated in preclinical and clinical investigations of molecular targeted therapies in HCC.

1. **Ligand–membrane receptor signaling pathways**

Epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet–derived growth factor (PDGF), and insulin–like growth factor (IGF) I or II act as extracellular upstream ligands for cell proliferation. Binding of these growth factors with their corresponding transmembrane receptors, including epidermal growth factor receptor (EGFR) or Her2/Neu, vascular endothelial growth factor receptor (VEGFR), platelet–derived growth factor receptor (PDGFR), and insulin–like growth factor receptor (IGF) I or II, leads to the progressive activation of the intracellular Ras/mitogen–activated protein kinase (MAPK) signaling pathway and the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, by triggering tyrosine kinase activity. Among these ligand–receptor signaling pathways, VEGFR and PDGFR, together with angiopoietin–2, me-

Table 1. Molecular targets and relevant therapeutic compounds on preclinical and clinical development in hepatocellular carcinoma

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EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet–derived growth factor receptor; IGF, insulin–like growth factor receptor; HGF, hepatocyte growth factor; MET, mesenchymal–epithelial transition factor; MAPK, mitogen–activated protein kinase; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; TRAIL, tumor necrosis factor–related apoptosis–inducing ligand.
mediate the angiogenesis, which has a pivotal role in human cancer, through the activation of different pathways in the tumor and endothelium. There are several ongoing preclinical or clinical trials focusing on the ligand-receptor signaling pathway in hepatocellular carcinoma. Cetuximab, trastuzumab, and bevacizumab are monoclonal antibodies against EGFR, Her2/Neu and VEGFR, respectively. Sorafenib, sunitinib, gefitinib, erlotinib, and lapatinib reciprocally react against VEGFR, PDGFR, EGFR, Her2/Neu as tyrosine kinase inhibitors.

The Wnt/β-catenin signaling pathway plays a critical role in hepatocarcinogenesis. The signal is initiated from the binding of Wnt ligands with the Frizzled receptors, which means an extracellular stimulation for the intracellular uncoupling of β-catenin from E-cadherin, which subsequently leads to translocation of β-catenin into the nucleus, where it regulates specific oncogenes responsible for cell differentiation and cell survival. The disrupted activation of this cascade has been shown in about 30% of HCC cases, particularly in HCV-related HCCs. However, until now, no effective molecule for blockage of this pathway has been identified, though there are several current preclinical trials under investigation.

The Hedgehog signaling pathway is implicated in a portion of human HCCs. Overexpression of this signal has been recently observed in more than half of human HCCs. After the binding of Hedgehog ligands with the membrane receptors, the consequent activation of a Smo molecule triggers the up-regulation of Gli-related target genes involved in cell differentiation and cell regeneration.

2. Intracellular signaling pathways

The Ras/MAPK signaling pathway consists of a sequential cascade from the phosphorylated activation of Ras, Raf, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK) and ERK, which have serine-threonine kinase activity. A functional activation of this pathway is induced from the aberrant activation of upstream tyrosine kinase receptor signals as mentioned above and inactivation of tumor suppressor genes as NO-RE1A or RAFSS1A, resulting in cell proliferation and cell survival. Most cases of HCC in the advanced stage are on activated status of the Ras/MAPK signaling pathway. Sorafenib, an outstanding breakthrough in the field of molecular therapy of cancer, exerts its antiangiogenic and antiproliferative effect by targeting not only the tyrosine kinase receptor VEGFR and PDGFR but also the Raf kinase, especially B-Raf, of the Ras/MAPK signaling cascade.

The PI3K/Akt/mTOR signaling pathway is also activated by the dysregulated activation of several tyrosine kinase receptor signals including mesenchymal-epithelial transition factor (MET) or through the functional loss of tumor suppressor genes such as PTEN. The aberrant activation of this pathway is responsible for cell growth and cell proliferation linked to the cell cycle allowing a progression from the G1 to S phase, and subsequent reaction against the apoptotic signals. The activation of this pathway is known to occur in 30–50% of HCC cases and is reported to be related to poor prognosis. The kinase mTOR, a downstream kinase of this pathway, is also closely related to the translational regulation of genes, such as c-myc, cyclin D1, and hypoxia-inducible factor.
(HIF)-1α, involved in angiogenesis.\textsuperscript{52} Rapamycin, an antibiotic with immunosuppressive activity, blocks mTOR and in turn leads to preventing HCC cell lines or experimental models of HCC from being induced to uncontrolled proliferation and aberrant differentiation.\textsuperscript{53,54} Everolimus and temsirolimus as rapamycin analogs, which were approved for the treatment of rectal cancer and immunosuppressive treatment after liver transplantation, are recently being investigated under phase I/II clinical trials.\textsuperscript{55,56}

3. RNA interference pathway

RNA interference (RNAi), a phenomenon of post-transcriptional gene silencing, interferes with the delivery of genetic information by sequence-specific double-stranded RNA (dsRNA).\textsuperscript{57} Small noncoding RNAs guiding the RNAi pathway, such as microRNA (miRNA), small interfering RNA (siRNA) and short hairpin RNA (shRNA), can act as micromolecules with tumor suppressor or oncogenic properties depending on their corresponding target genes.\textsuperscript{58} Although there was several miRNAs that were up-regulated or downregulated in HCC compared with surrounding nontumoral tissue,\textsuperscript{59-61} the role of these micromolecules in hepatocarcinogenesis remains to be seen. In the near future, a rational therapeutic for HCC will be provided by the clinical application of chemically synthesized small RNAs interfering with carcinogenic biomarkers.

4. Apoptotic pathways

Apoptosis, one of the programmed cell death regulated genetically, usually occurs when cells are deprived of its own proper function by a reversible damage. Dysregulation of apoptosis induces the destruction of cellular homeostasis resulting in tumor development and growth. Bel-2 antisense and proapoptotic receptor agonists such as recombinant Apo2L/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which target the intrinsic (mitochondria-mediated) pathway and the extrinsic (receptor-mediated) pathway, respectively, are being investigated in preclinical and experimental studies.\textsuperscript{62}

**Clinical trials of sorafenib as a molecular targeted agent**

A comprehensive meta-analysis assessing the outcomes of randomized clinical trials of systemic chemotherapeutic agents in patients with unresectable advanced HCC revealed that doxorubicin or platinum-based systemic or intraarterial chemotherapies, tamoxifen or antiandrogen-based hormonal therapies, and interferon alfa-oriented immunotherapy were all ineffective or minimally effective.\textsuperscript{63-65} Until now, authorized institutes and practical guidelines have not officially approved any drug for advanced HCC. The lack of clinical efficacy of systemic chemotherapies has encouraged clinical hepatologists to try the clinical application of molecularly targeted compounds to advanced HCC.

Sorafenib is an orally active multikinase inhibitor that inhibits both the membrane receptor tyrosine kinase and intracellular serine-threonine kinase responsible for tumor cell proliferation and tumor angiogenesis.\textsuperscript{44} In vitro, this multikinase inhibitor has induced the dose-dependent inhibition of cell proliferation and apoptotic cell death in human HCC cell lines.\textsuperscript{35} This compound has also demonstrated the anti-tumor activity in a murine xenograft model of
human HCC in dose-dependent manner.\textsuperscript{26}

Recently, phase III, multicenter, randomized, double-blind, placebo-controlled trials testing the multikinase inhibitor sorafenib have been performed in target populations of geographically different regions: Europe/America/Australasia and Asia-Pacific.\textsuperscript{12,13} The clinical results of these two trials, the Sorafenib Hepatocellular Carcinoma Assessment Randomised Protocol (SHARP) trial and the Asia-Pacific trial, showed that median overall survival (OS) and time to progression (TTP) were significantly longer for patients treated with sorafenib than for those given a placebo. In the SHARP trial,\textsuperscript{12} especially, the study was stopped at the second planned interim analysis, when 321 death had occurred, due to the definite survival advantage observed in the sorafenib group. This trial also identified eight baseline characteristics as prognostic indicators for overall survival: Eastern Cooperative Oncology Group (ECOG) performance status, macroscopic vascular invasion, tumor burden, Child–Pugh classification, alpha-fetoprotein, albumin, alkaline phosphatase, and total bilirubin. Even after subgrouping the enrolled HCC patients according to these prognostic factors, the clinical analysis showed a survival benefit for sorafenib over placebo. The Asia-Pacific trial\textsuperscript{13} was carried out to achieve regulatory approval of this drug in Asia-Pacific regions. Though designed in parallel with the SHARP trial, the Asia-Pacific trial was performed in the following clinical points: randomization in a 2:1 ratio to receive either sorafenib or placebo and absence of primary efficacy endpoint.

Drug-related adverse effects, which were hand–foot skin reaction, diarrhea, alopecia, fatigue, rash or desquamation, anorexia, and nausea, roughly in the order of their incidence, were reported to be similar in both trials and were almost controllable through the conservative treatments with dose adjustment of the drug. There were cases of drug discontinuation that were mainly caused by poor compliance due to the disease progression itself but not drug-related serious adverse effects above grade 2. Comparing these two trials, the median OS and TTP were shorter in the sorafenib group of the Asia-Pacific trial than in the same group of the SHARP trial (6.5 vs 10.7 months, 2.8 vs 5.5 months, respectively). This modest difference in clinical outcomes between the two trials resulted from the difference of clinical characteristics and performance status of enrolled patients. The Asia-Pacific trial enrolled HCC patients with the more advanced Barcelona Clinic Liver Cancer (BCLC) staging system (advanced BCLC: 95\% vs 82\%), more extrahepatic involvement (69\% vs 51\%), and more poor performance status (1–2 ECOG: 74\% vs 46\%) than enrolled subjects in the SHARP trial.\textsuperscript{12,13}

Although these two international trials showed a definite clinical benefit and acceptable safety profile of sorafenib therapy in patients with advanced HCC, the therapeutic effects are thought to be limited. The survival gain of approximately 2–3 months is not sufficient to satisfy the medical desires of patients and clinicians despite its statistical significance. Both the SHARP and Asia-Pacific trials also failed to show a significant difference between the sorafenib and placebo groups in the median time to symptomatic progression,\textsuperscript{12,13} which means that sorafenib administration did not prevent the subjective progression of disease morbidity and did not improve the quality of life. Furthermore, the level of response measured according to the
Response Evaluation Criteria in Solid Tumors (RECIST) revealed that the complete and partial response rates were 0% and 2~3.3%, respectively, in both trials.¹²,¹³ According to another clinical study testing the clinical role of sorafenib, results have never shown a clinical impact on advanced HCC patients with Child–Pugh classification B or C of liver cirrhosis.⁶⁶ There is no evidence that it has a significant beneficial impact on clinical outcomes in advanced HCC with more deteriorated function of hepatic reserves. Further clinical investigations are needed to overcome these disappointing clinical aspects. Besides sorafenib monotherapy, combination therapy with sorafenib plus other molecular targeted compound or systemic chemotherapeutic agent are being tried in several clinical investigations for the treatment of patients with advanced HCC.

**Future perspectives**

The pathogenetic clarification of HCC at the molecular and cellular level is essential for the molecular targeting for management of advanced HCC. Although the recent introduction of sorafenib, along with its positive clinical results, is worth our attention, the field of molecular management of HCC is taking its first step. The therapeutic outcomes – the prolongation of median survival and time to progression for approximately 3 months compared with conservative treatment of advanced HCC – are not enough to satisfy clinical hepatologists. Further translational research should be continuously conducted for expanding the understanding of molecular complexity in HCC initiation and progression, resulting in more satisfying consequences in setting of clinical research through the profound knowledge of hepatocarcinogenesis.

In addition, molecular classification of HCC through the excavation of specific biomarkers related to biological behavior is also necessary for classifying patients with diverse drug responses into groups with unique biological homo- geneity, ultimately optimizing the individualization of molecular targeted therapy in HCC.¹⁶,¹⁷ In the future, clinical investigations are needed for assessing the role of molecular compounds as adjuvant therapy following previously established treatments such as resection, liver transplantation, percutaneous ablation therapy, and TACE, for identifying the clinical efficacy of combination therapy of chemotherapeutic agents and molecular compounds focusing on molecular multi-targets, and for analyzing the cost-effectiveness and safety of molecular targeted therapies in patients with advanced HCC.

요 약

간세포암증은 발생률에 있어서는 전 세계적으로 지역적인 차이를 보이나 암 사망률에 있어서는 폐 암증과 위암증에 이어 3위를 차지하는 악성 종양이다. 지난 수십 년 동안 근치적 치료가 불가능한 진행성 간세포암증 환자의 생존율을 향상시킬 수 있는 효과적인 전신항암치료법은 없었다. 그러나 최근 multikinase 억제제인 소라페닙(sorafenib)이 간세포암증 환자의 생존율을 유의하게 향상시키고 암증의 진행을 늦춘다는 연구 결과가 발표되면서 진행성 간세포암증에 대한 분자생물학적 표적치료가 주목을 받고 있다. 소라페닙은 세포 내 신호전달체계 중 Raf-1과 B-Raf serine-threonine kinase를 차단함으로써 알세포 증식을 억제하고, 세포막 수용체 중 혈관내피성장인자 수용체(vascular endothelial growth factor receptor, VEGFR)와 혈소판성장인자 수용체(platelet-derived growth factor receptor, PDGFR)를 차단함으로써 신생혈관형성
(angiogenesis)을 막을 수 있는 약물이다. 그러나 지금까지 발표된 소라페닙 연구의 결과들을 보면 임상적으로 만족할 만한 수준에 도달하지 않았으며, 또한 다양한 약물반응을 보이는 경우가 있어 임정 생물학적 반응과 연관을 보이는 표지자의 발굴을 통해 간세포암종을 분류하려는 시도가 진행되고 있다. 즉 같은 생물학적 동질성을 갖는 간세포암종의 분자생물학적 분류를 통해 궁극적으로 간세포암종 환자의 개별화된 맞춤형 분자생물학적 표적치료를 최적화할 수 있을 것으로 생각된다.

REFERENCES


