Current Topics

Current Research Trends in the Exploration of Therapeutic Targets for Liver Disease

Molecular Targeted Therapy for Hepatocellular Carcinoma: Present Status and Future Directions

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Received March 13, 2015

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most lethal neoplasm, causing an estimated 700000 deaths annually. Currently HCC has only one systemic molecular targeted therapy, the multi-kinase inhibitor, sorafenib. The standard-of-care for advanced liver cancer is limited because sorafenib can expand the median life expectancy of patients for only 1 year. Thus there is an urgent need to develop a novel molecular targeted therapy to improve therapeutic outcomes for HCC. HCCs are phenotypically and genetically heterogeneous tumors driven by diverse molecular mechanisms. However, HCCs exhibit certain common traits selected through genetic and epigenetic alterations. The identification of common molecular alterations may provide an opportunity to develop more effective anticancer treatment through targeted therapy. Recent studies in liver cancer biology have revealed a limited number of molecular targets responsible for initiating and maintaining dysregulated cell proliferation, including vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), c-mesenchymal-epithelial transition factor-1 (c-Met), mammalian target of rapamycin (mTOR) and histone deacetylases (HDACs). New treatments involving inhibitors targeting several of these critical pathways are in development. This review describes the current understanding of target pathways, ongoing clinical trials using HCC-targeted agents, and future directions in the treatment of HCC.

Key words hepatocellular carcinoma; targeted therapy; inhibitor; clinical trial

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed and third most lethal neoplasm, causing an estimated 700000 deaths annually worldwide.[1)] The incidence of HCC has doubled over the past two decades, especially in the United States, Europe, Japan and China. Likewise, South Korea has about 14000 patients annually with HCC. HCC is generated predominantly due to risk factors that include hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcohol abuse, obesity and type 2 diabetes, with geographic variations.[2)] About 75% of HCCs are closely associated with chronic inflammation, caused by viral hepatitis in which continuous inflammation and hepatocyte regeneration occur.[3)] These long-term processes may include the accumulation of genetic and/or epigenetic changes, alteration of the liver tumor microenvironment, and the generation of liver cancer stem cells (CSCs).[4)] According to the Barcelona Clinic Liver Cancer (BCLC) staging system, curative treatments such as surgical resection, liver transplantation, or radiofrequency ablation-percutaneous ethanol injection (RFA-PEI) treatment, are primarily suitable for asymptomatic patients with very early and early (both stage A) HCCs, extending the median overall-survival (OS) by more than 60 months.[2,5)] The drug-eluting (DC)-BEAD transarterial chemoembolization (TACE) method is primarily curative for patients at an intermediate, multinodular stage B, with a median OS of about 20 months. Sorafenib is the only recommended treatment for patients with advanced HCC (stage C) involving invasive or extrahepatic tumors. The standard-of-care for advanced liver cancer can extend the median OS for about 11 months. Overall, the current treatment strategy for HCC can offer a survival benefit, particularly to patients in early stages.[6)] Of all the patients with HCC, only 30–40% are diagnosed as eligible for current curative treatments due to late diagnosis, underlying liver disease and lack of effective treatment options.[7)]

HCCs are phenotypically and genetically heterogeneous tumors driven by diverse molecular mechanisms. However, HCCs exhibit certain common traits selected through genomic and epigenetic alterations.[8,9)] The identification of common genomic alterations may provide an opportunity to develop anticancer treatment through targeted therapy.[10)] Sorafenib, which targets multi-kinases, is a current standard-of-care for advanced liver cancer, but extends the median life expectancy of patients by only 1 year. Therefore, there is an urgent need to develop a novel molecular targeted therapy for HCC. Recent advances in molecular pathogenesis studies have defined numerous molecular targets that are critical to HCC development, progression, and metastasis, including vascular endothelial growth factor receptor (VEGFR), epidermal growth factor

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receptor (EGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), c-mesenchymal-epithelial transition factor-1 (c-Met), mammalian target of rapamycin (mTOR) and histone deacetylases (HDACs).[11–13] New treatments of inhibitors targeting several of these critical pathways are in development. In this review, we describe the key genes and pathways involved in hepatocarcinogenesis, as well as the ongoing clinical trials using targeted inhibitors, and suggest new possibilities for developing therapeutic options for HCC.

2. KEY GENETIC ALTERATIONS

During the long-term process of tumor development, the pro-tumorigenic microenvironment generates genetic and epigenetic alterations that are essential for tumors to display their full neoplastic phenotype.[14] Namely, the eventual accumulation of molecular alterations in a subset of functional pathways initiates a biological environment to deregulate the homeostatic mechanism in normal cells. Hepatocarcinogenesis is a complex process driven mainly by chronic viral infections that alter the hepatic microenvironment.[5] A multistep sequence of genetic alterations disrupts normal hepatocellular processes, such as the proliferation, apoptosis, and maintenance of genomic integrity, which leads to inflammation, necrosis and regeneration, and eventually transforms the selected hepatocyte populations into dysplastic nodules that evolve into HCC.[15] Here we introduce the key molecular alterations known to play a role during HCC development; these are now being researched as molecular targeted agents in clinical trials.

Receptors for growth factors (VEGFR, FGFR, PDGFR) activate intracellular receptor tyrosine kinases (RTKs) and the downstream RAS/RAF/mitogen-activated protein extracellular kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway, and promote the growth, migration and morphogenesis of vascular endothelial cells, thus increasing vascular permeability (Fig. 1). By studying human HCC tissue and serum samples, the enhanced expression of VEGF is found to be correlated with aggressive behavior of HCC, resulting in a poor prognosis.[16] FGFR has been known to be involved in the growth, invasion and angiogenesis of HCC.[17] Although VEGF plays a major role in inducing tumor angiogenesis, crosstalk between VEGF and FGF signaling in angiogenesis is also necessary.[18] In the early stage of chronic hepatitis, the expression of PDGF was upregulated, which suggests that PDGF is related to the development of fibrosis in chronic hepatitis.[19] Since HCC is a highly vascularized tumor, the targeting of well-known angiogenic factors, such as VEGF, FGF, and PDGF, is appealing for the molecular therapy of HCC.

As illustrated in Fig. 1, the phosphatidyl inositol 3-kinase (PI3K)/AKT/mTOR pathway functionally interacts with VEGFR, PDGFR, and EGFR to exert cellular processes, including cell proliferation and survival. This pathway is often targeted in therapeutic intervention in HCC because activating it can cause aggressive tumor behavior and a reduction in

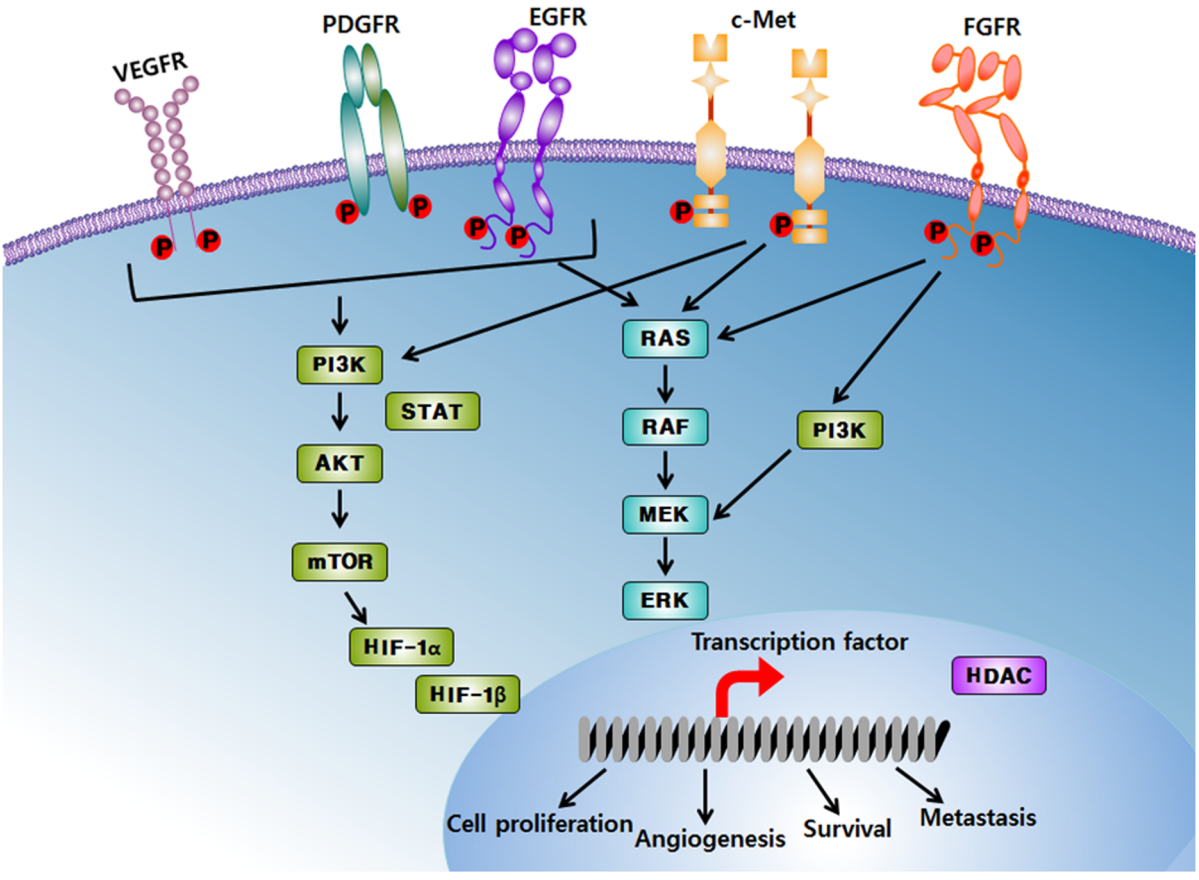

Fig. 1. Schematic Diagram of Key Functional Pathways and Their Roles in HCC Development

During hepatocarcinogenesis, the oncogenic pathways, such as VEGF, PDGF, EGF, c-Met, and FGF signaling, are activated to promote cell proliferation, invasion, and metastasis.

survival.[20] The RAS/RAF/MEK/ERK pathway, continuously activated in HCC, controls many essential cellular processes by interacting with EGFR, c-Met, and FGFR.[21] The hepatocyte growth factor (HGF)/c-mesenchymal-epithelial transition factor-1 (c-MET) pathway is associated with angiogenesis, invasion, and tumor growth in many types of cancer.[22] c-MET is a tyrosine kinase receptor which is induced by its ligand, HGF. Activation of c-MET eventually leads to activation of the downstream effector molecules, PI3K and ERK. Moreover, in HCC patients, a deregulated c-MET receptor protein generally results in a poor prognosis.[23,24] Thus targeting the c-MET receptor could be a promising treatment for HCC.

Meanwhile, in the course of studies of gene expression signatures as predictors of survival rates for HCC patients, it was identified that the expression patterns of a limited number of genes were significantly correlated with disease prognosis, providing a new molecular insight into the pathogenesis of HCC.[25] Representatively, the HDAC2 gene involved in histone modification was highly expressed in the tumor tissue of a low survival subgroup of HCC patients. Histone deacetylases (HDACs) belong to a family of chromatin-modifying enzymes which repress gene expression by removing acetyl groups from histone substrates, and are thus involved in the epigenetic regulation of the cell cycle, apoptosis and differentiation.[26] Given the functional significance of HDACs as epigenetic regulators of a large number of genes and signaling cascades in human cancer, the pharmacological targeting of HDACs is emerging as a promising anti-cancer strategy.[27] Molecular targeting of HDAC2 with target-specific RNA interference (RNAi) caused the inhibition of HCC cell growth through deregulating a limited number of genes, including HDAC-regulated genes that are involved in controlling the cell cycle, apoptosis and lipid metabolism.[13]

3. MOLECULAR TARGETED AGENTS IN CLINICAL TRIALS

Currently, most clinical investigations for HCC are utilizing agents that target various growth factors and their receptors, as summarized in Table 1. In this study we mainly describe molecular targeted agents used during phase III with advanced HCC patients.

Sorafenib Sorafenib is a multi-kinase inhibitor that targets Raf-1, B-Raf, VEGFRs 1, 2, and 3 and PDGFR-β.[28,29] In the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial, sorafenib significantly increased the overall survival (OS) of HCC patients from 7.9 to 10.7 months when compared to a placebo (hazard ratio [HR] in the sorafenib group, 0.69; 95% confidence interval [CI], 0.55–0.87; $p<0.001$).[30] Sorafenib was the first agent developed and used in the systemic therapy of HCC, demonstrating an OS benefit for patients with advanced HCC, and its approval made a breakthrough in terms of the development of molecular targeted therapy for advanced HCC. Subsequently, sorafenib was applied to 271 patients in the Asia-Pacific region with advanced HCC. The median OS was 6.5 months in the sorafenib group and 4.2 months in the placebo group (HR, 0.68; 95% CI, 0.50–0.93; $p<0.014$).[31]

Brivanib Brivanib is an orally active tyrosine kinase inhibitor targeting the FGF and VEGF signaling pathways, both of which are associated with HCC pathogenesis.[32] In a randomized phase III clinical trial, although it did not meet its primary endpoint of OS in a non-inferiority trial for brivanib *versus* sorafenib, brivanib treatment yielded a 9.5 month OS, showing similar antitumor activity to sorafenib (9.9 months of OS) (HR, 1.06; 95% CI, 0.93–1.22; $p<0.373$).[33] In the second-line setting, it was proven that brivanib treatment did not improve the OS of patients who were refractory or intolerant to sorafenib (HR, 0.56; 95% CI, 0.42–0.76; $p<0.001$).[34]

Table 1. List of Molecular Targeted Agents Used for Clinical Trials against HCC

Agents	Main target	Phase	Reference
Sorafenib	VEGFR, PDGFR, Raf	3	30, 31
Brivanib	VEGFR2, FGFR1	3	33, 34
Linifanib	VEGFR, PDGFR	3	
Sunitinib	VEGFR, PDGFR, c-KIT, RET	3	35
Erlotinib	EGFR	3	38
Everolimus	mTOR	3	40
Ramucirumab	VEGFR	3	
Lenvatinib	VEGFR, PDGFR, FGFR, RET, SCFR	3	
Regorafenib	VEGFR, PDGFR, BRAFFGFR, KIT, RET	3	
Cabozantinib	c-MET, VEGFR, RET	3	
Gefitinib	EGFR	2	
Bevacizumab	VEGF	2	
Cediranib	VEGFR, PDGFR, c-KIT	2	
BIBF-1120	VEGFR, PDGFR, FGFR	2	
E-7080	VEGFR, FGFR, PDGFR, c-KIT	2	
TSU-68	VEGFR2, FGFR, PDGFR	2	
XL-184	VEGFR2, MET, RET	2	
Vandetanib	VEGFR, EGFR	2	
Cetuximab	EGFR	2	
Tivantinib	c-MET	2	43
MEDI-575	PDGFR	1	
BAY73-4506	VEGFR, PDGFR, FGFR-1Raf, RET, c-KIT	1	

Sunitinib Sunitinib is an oral and multitargeted tyrosine kinase inhibitor targeting all VEGFRs, PDGFRs, c-kit, and Fms-like tyrosine receptor kinases (Flt) 3 and rearranged during transfection (RET) genes. A large phase III trial with a total of 1074 HCC patients was conducted to evaluate whether sunitinib had superior efficacy relative to sorafenib. The result showed the median OS with sunitinib was not superior but instead significantly inferior to sorafenib (7.9 *versus* 10.2 months; HR, 1.30; one-sided $p<0.9990$; two-sided $p<0.0014$). The median OS with sunitinib was similar among Asian and HBV-infected patients, but shorter in HCV-infected patients (9.2 *versus* 17.6 months; HR, 1.52; one-sided $p<0.9835$).[35]

Erlotinib Erlotinib, an orally active inhibitor of EGFR tyrosine kinase, showed modest antitumor activity in a phase II clinical trial but offered a promising OS benefit of about 11–13 months for patients with unresectable HCC.[36,37] More recently, in a phase III SEARCH trial, patients with advanced HCC were randomized to take either sorafenib plus erlotinib or sorafeib plus a placebo to test the combinatory effect. The median OS was 9.5 months with the combinatory treatment and 8.5 months with sorafenib only (HR 0.92; 95% CI 0.78–1.1; $p<0.2$), indicating that the combination of sorafenib and erlotinib did not significantly improve the OS of patients with advanced HCC.[38]

Everolimus Everolimus is an mTOR inhibitor that has been demonstrated to prevent tumor progression and to improve survival in preclinical HCC models.[39] Recently, in a phase III study, the first Everolimus for liver cancer evaluation (EVOLVE-1) was conducted to assess the efficacy of everolimus in patients with advanced HCC for whom sorafenib treatment had failed. No significant difference in median OS was observed between the everolimus and placebo treatment groups (HR, 1.05; 95% CI, 0.86–1.27; $p<0.68$; median OS, 7.6 months with everolimus, 7.3 months with placebo).[40]

Tivantinib Tivantinib, a candidate anticancer agent for HCC patients that selectively targets c-MET, is mainly metabolized by CYP2C19.[41] A phase I study was recently completed with 28 Japanese HCC patients who failed with sorafenib, to evaluate the safety, tolerability, and pharmacokinetics of oral tivantinib as a single agent. As a result, it was found that 120 mg twice daily (BID) of tivantinib could be recommended for HCC patients regardless of CYP2C19 phenotype.[42] In a randomized phase II trial, tivantinib and a placebo were compared for a second-line treatment. The overall analysis showed that patients with MET-high tumors who received tivantinib had Median OS of 7.2 months, compared to 3.8 months for MET-high patients who received the placebo (HR, 0.38; 95% CI, 0.18–0.81; $p<0.01$).[43]

4. FUTURE DIRECTIONS

Over the past few decades, there have been substantial advances in developing molecular targeted therapy for HCC by elucidating the diverse genetic alterations driving HCC initiation, progression, invasion, and metastasis. However, neither targeted agents nor regimens other than sorafenib have yet significantly improved the OS in HCC patients with advanced disease status, mainly due to a lack of efficacy, flaws in trial design, and toxicity.[12] HCC is a highly heterogeneous disease driven by its complex pathogenesis, which involves diverse genetic dysregulations in numerous functional pathways. Drugs designed to act against individual molecular targets may not be able to effectively combat HCC's multigenicity. Combinatorial trials with two or three drugs that impact multiple targets simultaneously may lead to better treatments to control the complex disease system, and may be the next standard of care for HCC patients. In fact, further improvements in targeted therapy for HCC may require a better understanding of the various molecular events driving HCC progression, as well as identification of the biomarkers to classify responder and non-responder HCC patient groups for targeted agents. In addition to genetic changes, it was recently discovered that epigenetic alterations, such as DNA methylation, histone modifications and/or non-coding microRNA patterns, are also involved to hepatocarcinogenesis; thus, novel approaches to developing epigenetic therapy are under active investigation.[44] Unlike genetic events, aberrant signaling in epigenetic regulations are reversible; therefore, targeting the epigenetic machinery of HCC is a strong potential alternative option for single or combinatorial trials with existing targeted agents (*e.g.* sorafenib) in the treatment of HCC.

Targeting the tumor microenvironment is the next issue. While the existence of cells with self-renewing capacity in HCC is well supported, the origin of these cells remains uncertain. Recently, growing evidence supports the novel notion that tumor initiation can be driven by a CSC subset, which is responsible for tumor persistence, relapse, metastasis, chemoresistance and radioresistance. Only a subpopulation of cells within liver cancer harbors genuine tumorigenic potential. Classical therapeutic regimens predominantly target proliferating cells, which are unlikely to be CSCs. Similarly, new generation therapies (*e.g.* sorafenib) do not seem to target CSCs, as evidenced by frequent tumor relapse and resistance after therapy. Identification and characterization of signaling pathways and biomarkers associated with CSC biology are therefore priorities for developing new paradigms of molecular cancer therapeutics in the treatment of HCC. Experimental evidence suggests that any hepatic lineage cell (*i.e.*, hepatocytes/cholangiocytes, progenitor cells, stem cells) can obtain stem cell properties and become a CSC upon acquiring genetic and epigenetic alterations in diverse signaling pathways. Many of the identified regulatory pathways (bone morphogenic protein (BMP), Wnt, TGF-β, MYC, p53, IL-6/STAT3, *etc.*) are known to be involved in stem cell maintenance as well as in self-renewal and pluripotency.[4,45] The disruption of these functionally overlapping pathways showed a frequent association with the prognosis in liver cancer. Hepatic CSCs are clearly heterogeneous and may therefore contribute to the morphological and biological heterogeneity characteristic of HCC. The CSCs not only initiate HCC, but also drive distant metastasis and relapse after therapy, thereby establishing CSCs as important therapeutic targets. A better understanding of the molecular pathway(s) involved in the generation of tumorigenic CSCs in the liver microenvironment will provide useful information for developing novel treatment strategies against this deadly disease.

Acknowledgments This work was supported by Grants from the Korean Health Technology R&D Project, Ministry of Health & Welfare (A121982), the Bio & Medical Technology Development Program, Ministry of Science, ICT & Future Planning (NRF-2012M3A9C7050149), and the

Translational Research Institute for Novel Drug (TREND) Program, Ministry of Science, ICT & Future Planning (NRF-2012M3A9C1053340), Republic of Korea. I apologize for not stating more acknowledgments for the many excellent works contributed to this field due to space limitation.

Conflict of Interest The authors declare no conflict of interest.

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