Hepatocellular Carcinoma: Etiology and Current and Future Drugs

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Hepatocellular carcinoma (HCC) is swiftly increasing in prevalence globally with a high mortality rate. The progression of HCC in patients is induced with advanced fibrosis, mainly cirrhosis, and hepatitis. The absence of proper preventive or curative treatment methods encouraged extensive research against HCC to develop new therapeutic strategies. The Food and Drug Administration–approved Nexavar (sorafenib) is used in the treatment of patients with unresectable HCC. In 2017, Stivarga (regorafenib) and Opdivo (nivolumab) got approved for patients with HCC after being treated with sorafenib, and in 2018, Lenvima (lenvatinib) got approved for patients with unresectable HCC. But, owing to the rapid drug resistance development and toxicities, these treatment options are not completely satisfactory. Therefore, there is an urgent need for new systemic combination therapies that target different signaling mechanisms, thereby decreasing the prospect of cancer cells developing resistance to treatment. In this review, HCC etiology and new therapeutic strategies that include currently approved drugs and other potential candidates of HCC such as Milciclib, palbociclib, galunisertib, ipafricept, and ramucirumab are evaluated. (J Clin Exp Hepatol 2019;9:221–232)

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Abbreviations: FDA: Food and Drug Administration; HCC: Hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis B virus; HDV: hepatitis D virus; Rb: retinoblastoma protein; HBsAg: HBV surface antigen; HIV: human immunodeficiency virus; HBcAg: hepatitis B core antibody; PDGFR: platelet-derived growth factor receptor; CTGF: connective tissue growth factor; EMT: Epithelial–mesenchymal transition; ECM: extracellular matrix; TGF 1: transforming growth factor-1; CTL: cytotoxic T lymphocyte; Treg: regulatory T cells; NK: natural killer; NKT: natural killer T cell; MDSC: myeloid-derived suppressor cell; BMI: body mass index; NASH: nonalcoholic steatohepatitis; PAPSS1: 3′-phosphoadenosine 5′-phosphosulfate synthase 1; TK: tyrosine kinase; CDK: cyclin-dependent kinase; TKI: Tyrosine kinase inhibitor; ATP: adenosine 5′-triphosphate; IGFR: insulin-like growth factor; SHP1: src homology 2 domain–containing phosphatase 1; PD-L1: programmed death ligand1; GFG: fibroblast growth factor; SCF: stem cell factor; OS: overall survival; PFS: progression-free survival; TACE: transarterial chemoembolization; TRKA: tropomyosin receptor kinase A; AMPK: AMP-activated protein kinase; RFA: radiofrequency ablation; ORR: objective response rate; PD1: programmed cell death protein 1; EGFR: epidermal growth factor receptor; EFGR: endothelial growth factor receptor; PI3K: phosphoinositide 3-kinases; STAT3: signal transducer and activator of transcription 3; VEGF: vascular endothelial growth factor; PEDF: pigment epithelium-derived factor; HIF: hypoxia-inducible factor; VEGFR: vascular endothelial growth factor receptor; bFGF: basic fibroblast growth factor; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; JAK: janus kinase; BMF: Bcl2 modifying factor; PUMA: p53 upregulated modulator of apoptosis; PTEN: phosphatase and tensin homolog; AMP: adenosine monophosphate; CTLA: cytotoxic T-lymphocyte-associated protein

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PREVALENCE

Hepatocellular carcinoma (HCC) is the most prevalent malignancy of the liver, which is considered the second leading cause of cancer death in the US. It is considered as the fifth most detected cancer in men and the seventh most detected cancer in women in the USA and the third most leading cause of cancer death worldwide.[1] In many parts of the world including Europe, North America, and Latin America, the rate of liver cancer is increasing by 3.1% each year from 2008 to 2012, as reported by the database of the National Cancer Institute in the US.[2] According to recent reports, the highest incidence rate of liver cancer in the world occurs in Africa and Asia. Hepatitis B virus (HBV) and hepatitis C virus (HCV) account for approximately 80% of HCC cases, of which HBV accounts for 50–80% of cases of virus-associated HCC, whereas HCV is known to be responsible for 10–25%.[3,4]

HCC is diagnosed more commonly in men than in women possibly due to a higher prevalence of HBV, HCV, and alcohol consumption in males that translate into increased carcinogenicity.[1] Other factors that contribute to the escalation of HCC include increased occurrence of obesity, aflatoxin exposure, and nonalcoholic fatty liver disease around the world.[1]

ETIOLOGY

Several factors associated with the etiology of HCC have a direct influence on disease progression and on the characteristic of patients.[4] The greatest global HCC incidence has been reported from sub-Saharan Eastern and Western Africa, Mongolia, China, and Asia-pacific regions.[5] However,

the pervasiveness of HCC is lower in developed countries excluding France, Japan, and Italy. HBV, HCV, and hepatitis D virus (HDV) have a strong link with the progression of HCC; therefore, the global incidence of HCC mirrors the occurrence of these infectious viral diseases. Liver cirrhosis progresses to HCC in ~80–90% of the cases.[6] The paramount risk element for HCC to flourish is cirrhosis, and HCV and HBV are regarded as the crucial causes of cirrhosis. As a matter of fact, cirrhosis happens when hepatocytes undergo necrosis resulting in fibrosis-forming scar tissue in cirrhosis.[7] It is well known that HCC is the mainspring of HBV infection.[8] To a large extent, HCC linked to HBV occurs in patients who are suffering from HBV infection nearly all their lives, namely chronic hepatitis B.[8]

Hepatitis B virus

HBV infections may develop into HCC in the presence or absence of cirrhosis due to the genetic mutation induced by HBV.[9] The HBV genetic material infiltrates normal hepatocytes and interrupts their function, which result in cancerous cells. Fragments of the HBV genome are often detected in these hepatocarcinoma cells.[10] HBV has a partly double-stranded DNA that incorporates virus associated to the Hepadnaviridae family.[11] HCC is induced by this virus through both direct and indirect pathways. HBV infection in the liver is the cause of hepatocyte lesion and chronic necroinflammation ensuing hepatocyte proliferation, fibrosis, and cirrhosis.[12–14] The unceasing regeneration in cirrhosis persuades hepatocyte multiplication, turnover, and buildup of mutations in the host genome, resulting in genetic changes, chromosomal rearrangements, inactivation of tumor suppressor genes, and activation of oncogenes.[14] Nevertheless, in the absence of cirrhosis, HBV can also induce HCC.[4] However, HBV may behave as a mutagenic factor by resulting in secondary chromosomal rearrangement and escalate genomic mutability via integrating its DNA into host cells.[4]

While most HCC cases evolve in cirrhotic livers, a crucial fragment of HBV-associated HCC may take place in the setting of chronic hepatitis B in the lack of liver cirrhosis. The fact that a lower rate of cirrhosis in HBV-associated HCC is analogous to other etiologies, thus argues for a more direct role of HBV in the tumor formation process. Furthermore, different gene expression profiles have been diagnosed in the nontumoral livers of chronic HBV carriers. For instance, activating expression of genes linked in proapoptotic, inflammatory, and DNA repair responses indicate definite pathways activated by chronic hepatitis B.[15,16] Hepatitis B virus X (HBx) gene plays an essential role in HBV-associated HCC development. In addition, HBx functions as an activator for an enormous variety of viral promoters. Hence, HBx gene expression is of foremost significance for viral reproduction within living cells.[17–22] HBx protein of HBV or NS5A protein of HCV can cause chronic infections that trigger the PI3K/Akt/STAT3 pathway in tumor cells.[23]

Hepatitis C virus

HCV is associated with the *Hepacivirus* genus of Flaviviridae descent, and it infects approximately 170 million people globally per year.[24] As compared to uninfected subjects, a 15- to 20-fold increased threat for HCC exists in HCV-infected individuals.[24] Throughout the extent of 30 years of persistent infection, the momentum of HCC in cohort studies of HCV-affected persons extends from 1% to 3%. After HCV-associated cirrhosis is confirmed, HCC evolves at a yearly rate from 1% to 8% at an average of 3.5%.[24,25] Unlike HBV that can integrate into the host genome resulting in the direct carcinogenic activity, HCV is known to be an RNA virus with the restricted incorporation of its genetic information into the host genome.[26] Consequently, the carcinogenic prospective of HCV is linked to indirect mechanisms.[26] Although HCV elimination can play a role in preventing the progression of HCC, other factors that play a major role in HCC progression are iron overload, oxidative stress, endoplasmic reticulum stress, steatosis in hepatocytes, and inflammation.[27]

Nevertheless, HCV may also directly progress to HCC by amending various host regulatory pathways that are required in epithelial–mesenchymal transition, angiogenesis, apoptosis, proliferation, and DNA repair. Recent studies have identified direct targets of HCV proteins such as retinoblastoma protein (Rb) that is responsible to restrain cell proliferation primarily by suppressing the activation of E2F, a transcription factor required for S-phase ingression in the cell cycle.[28–32]

Dual infection

There are several salient similarities shared by HBV and HCV such as the modes of transmission, large diffusion globally, and the ability to trigger a chronic infection that may progress to cirrhosis and hepatocellular carcinoma.[33] Gathered epidemiological data suggest that coinfection with HBV and HCV escalates the risk for the progression of HCC. A massive body of data revealed that the pervasiveness of esoteric HBV infection that is the enduring persistence of HBV genomes in person negative for HBV surface antigen (HBsAg) is specifically raised in HCV individuals.[34–36] Recent studies have demonstrated that coinfection has long-term acute evolution as compared to HBV or HCV monoinfection. Furthermore, dual infection is linked with an elevated risk of development of fibrosis and the progression of cirrhosis and is a discrete predictor of HCC progression.[37,38] Thus, coinfection with HBV or HCV is an intricate clinical/virological form[39] that seems to be linked with the various manifestation of chronic liver disease, and it is a major risk factor for HCC progression.[40,41]

The human immunodeficiency virus (HIV) is considered as another major modulator of HCC. Studies have revealed that HIV coinfection can hasten the clinical progression of chronic HBV or HCV infection and enlarge the risk of liver cirrhosis and HCC.[42,43] The impact of HBV or HCV on HIV are, however, contentious, and some studies have described that HIV-positive patients coinfected with HCV and/or HBV have the more swift development of AIDS and associated death than patients without coinfection.[44] Furthermore, HIV and HBV share a similar course of transmission as the prevalence of anti–hepatitis B core antibody (HBcAb) and HBsAg in HIV-positive patients are exceptionally elevated. Discrete, usually vital, virological profiles may be perceived that is particularly associated with the proceedings of either one or both the viruses over time.[45] For the accurate diagnosis and therapeutic approach, it is obligatory to perform a cautious longitudinal assessment of the HBV and HCV titers.

Patient heterogeneity

Patient heterogeneity is a part of the natural alterations that can be assigned to the attributes of those patients.[46,47] Interpatient heterogeneity is described by the discrepancy of tumor cell populations within patients.[48,49] Hepatocellular carcinoma has diverse modifications that rely on tumor size and histological grade. Recent studies demonstrated that HCCs approximately 1.0 cm in size have artery-like vessels that are not properly grown with vague capillarization of the blood expanse and the main portal supply within cancerous nodules.[48] At distinct phases of tumor development, angiogenic shifts come from the balance between proangiogenic and antiangiogenic elements. Hence, angiogenic heterogeneity is related to angiogenic molecules such as VEGF, PEDF, and HIF-1 alpha. Therapy against angiogenic elements is crucial in restraining the recurrence in patients with HCC.[50] There are various antiangiogenesis targets such as VEGF, VEGFR, bFGF, platelet-derived growth factor receptor (PDGFR), and angiopoietin Ang-1 and Ang-2. A Food and Drug Administration (FDA)–approved kinase inhibitor, Sorafenib, could lessen the expression of VEGFR-2, VEGFR-3, and PDGFR.[51] The angiogenic heterogeneity of HCC is required to be taken into deliberation because angiogenic elements could be distinct among different tumor sizes and time span throughout the course of hepatocarcinogenesis.[50]

The connective tissue growth factor (CTGF) is overexpressed in individuals with HCC, but it is also known that the downregulation of CTGF could hamper HCC development.[52] It could potentially be a future therapeutic target for HCC treatment. Epithelial–mesenchymal transition (EMT) is a vital course of action in hepatocarcinogenesis, which includes the association between cells and extracellular matrix (ECM) mediated by transforming growth factor-1 (TGF 1) or PDGFR signaling. It is an onerous point to inhibit ECM proteins due to several ECM proteins and intricate mechanisms, and thus, it is important to consider it for target therapy.[53] The immune microenvironment in HCC also appears to be heterogeneous. Cell types enclosed within surrounding tumors incorporate CD8+ cytotoxic T lymphocytes (CTLs), regulatory T cells (Treg), natural killer (NK) cells, natural killer T (NKT) cells, and myeloid-derived suppressor cells (MDSCs).[54] The cells can be involved in developing or inhibiting HCC development.[55] The development of immunotherapy requires a comprehension of the heterogeneous microenvironment, regulation of cytokines at various stages of HCC, the functional activity of CTLs and NK cells, etc.[54] The heterogeneity of HCC cells comes from distinctive gene expression and genetic variations that modify signaling pathways and protein function.[49] In one study, it was found that intratumor heterogeneity was detectable in 87% of HCC cases. The same study also suggested that on the basis of tumor morphology alone, 26% of the cases were heterogenic.[56]

Various kinase inhibitors have been under development or reviewed for their clinical significance. Besides, it is important to develop a novel therapy averse to drug-resistant cancer stem cells. For subsequent clinical research design, it is obligatory to think about how to remove the confounding effects from genetic interpatient and intratumor heterogeneity. Targeting the heterogeneity of cancer cells/cancer stem cells, angiogenesis, invasion, and immune reactions might be a promising strategy for individual personal treatment options.

Aflatoxin

Aflatoxins are naturally prevailing subsidiary metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*.[57] It is known to be a food contaminant and a well-known human hepatocarcinogen which is a prime agent in the pathologic process of HCC.[58] Aflatoxins may be present in a broad range of food items that include peanuts, meat, milk, oilseeds, corn, and dried fruits. There are numerous factors that influence the development of *Aspergillus* and the amount of aflatoxin contamination in foods. One of the factors that increase the susceptibility of plants to *Aspergillus* resulting in aflatoxin contamination is drought stress.[58] Once consumed, AFB1 is metabolized to a functional transitional compound, AFB1-exo-8,9-epoxide, that can attach to DNA.[59] AFB1 generates a mutation at serine 249 in the tumor suppressor p53 that was diagnosed in 30–60% of HCC samples collected from people in the aflatoxin-prevalent region, most of whom were suffering from HBV disease.[60] Assays have been established to diagnose specific aflatoxin-related DNA mutations in tissues

and to calculate metabolites in urine and AFB1–albumin adducts in serum.[59]

Metabolic syndrome

As compared to patients without metabolic syndrome, it is reported that those with metabolic syndrome such as obesity and diabetes have a higher incidence of HCC.[1,61]

Obesity

A study of liver cancer manifestation indicated that a body mass index (BMI) $\geq$25 kg/m^2 is implicated with a higher risk of developing primary liver cancer.[62] The connection between liver cancer and BMI is independent of alcohol ingestion, geographic location, and diabetic history. Nevertheless, the link between BMI and HCC is much greater in individuals with HCV infection than in individuals with HBV infection.[63] Obese males had a higher risk of primary liver cancer than obese females.[64] Moreover, people with nonalcoholic steatohepatitis (NASH) often have metabolic syndromes such as obesity, insulin resistance, and hyperlipidemia. The widespread presence of obesity and the series of hepatic histological damage are considered to be related with NASH.[65] However, obesity increases liver inflammation that leads to fibrosis and further progress toward NASH-related cirrhosis.[65]

Genetic factors

In addition to the well-established role of hepatitis virus infections and consumption of alcohol in the progression of HCC, various genetic aspects or syndromes also play a vital role.[66] Family-based studies proposed that a genetic locus on a 4th chromosome, encoding the candidate 3′-phosphoadenosine 5′-phosphosulfate synthase 1 (PAPSS1) gene, can regulate the risk of HCC in HBV-positive individuals in the Chinese community.[66] It has been established that the yearly prevalence of HCC is 4% in patients with hereditary hemochromatosis.[67] Moreover, it appears that concomitance of these genetic conditions with known HCC risk components such as viral hepatitis and alcoholism would increase their oncogenic potential. Thus, patients with familial genetic disorders of the liver should be frequently counseled to elude toxic and environmental damage to the liver.[67]

REGULATION OF KINASES IN HCC

Two major kinase types are dysregulated in HCC, namely the tyrosine kinases (TKs) and cyclin-dependent kinases (CDKs). These groups of kinases play a crucial role in cell growth and metabolism and are emerging targets for the treatment of HCC.

Tyrosine kinases

TKs are phosphorylating enzymes that take part in various cellular pathways. In general, TKs play a vital role in regulating growth factor signaling. The activation of TKs results in increased tumor cell proliferation and growth, has antiapoptotic effects, and stimulates angiogenesis. Protein kinases are involved in commencing tumorigenesis when they are activated by somatic mutations. Tyrosine kinase inhibitors (TKIs), as their name entails, bind TKs or suppress adenosine 5′-triphosphate (ATP) binding to interfere with signal transduction.[68–70]

TKIs are an essential class of target-defined therapy for a diverse range of malignancies in addition to HCC. TKIs possess antitumor activity and share a common mechanism of action by competitively inhibiting ATP binding at the catalytic domain of various oncogenic TKs. Although the inhibitors such as sorafenib, sunitinib, regorafenib, lenvatinib, palbociclib, and abemacicilib vary by their pharmacokinetic effects, subject-defined harmful outcomes and diversity of targeted kinases are considered as important factors that define the effectiveness of TKIs.[71] TKIs are categorized into three major categories. Nearly all the current TKIs are ATP-competitive inhibitors and are categorized as type I inhibitors. There are various problems that hinder the development of specific/particular TKIs of type 1 because of the immensely conservative ATP-binding sites in TK domains and an elevated rate of competition with intracellular ATP. Hence, TKIs might target other kinases, thus indicating that the antitumor effects are possible because of other signaling molecules. Moreover, TKIs that are non-ATP competitors are known as types II and III. These non-ATP competitors have conformation shifts causing structural changes in receptor TKs that result in the modification of TK domain in such a way that the TK domain reduces its kinase activity. In addition, these inhibitors can bind to the remaining part within the TK domain and impede tyrosine phosphorylation.[72,73] Numerous cellular functions that include differentiation, cell growth and survival, transduction of signals from membrane-bound tyrosine kinase receptors including vascular epidermal growth factor receptor (EGFR), insulin-like growth factor (IGFR), endothelial growth factor receptor (EFGR), and PDGFR to the cell nucleus via Ras/MAPK signaling pathway. Dysregulation of the Ras/MAPK pathway leads to improper cellular activities such as increased cell growth and differentiation, and eventually to cancer.

Cyclin-dependent kinases

A cell cycle is comprised of a series of interconnected biochemical pathways that are tightly regulated via checkpoints to ensure the passing of intact genetic material from one parent cell to the newly divided cells. Overall, a cell cycle has four phases, G1 or gap phase (cell growth), S or synthesis phase (DNA synthesis), G2 or second gap phase (prepares to divide), and M or mitosis phase (cell division). The cell cycle progression is driven by the centrally placed

CDKs, which are serine/threonine protein kinases that phosphorylate key substrates to promote DNA synthesis and mitotic progression. CDKs are normally present in molar excess but are inactive until bound by their cognate cyclin subunits. These subunits are tightly regulated at both the levels of synthesis and ubiquitin-dependent proteolysis.[74] CDKs are essential for the control of the cell cycle and to regulate apoptosis. Moreover, CDKs are found to be deregulated in most cancer cells. In HCC, there is an upregulation of CDKs via inactivation of CDK inhibitory proteins and through increasing levels of cyclins.[75–79] Studies demonstrate that CDKs are considered to have various functions as they are included in glucose homeostasis, mRNA regulation, and nerve cells differentiation.[79]

The CDKs consist of a family of about 20 members, and among them, the CDK1–CDK6 have been related to control of cell cycle, development, and homeostasis. To form an activated complex, CDKs get associated with a cyclin. The activity of CDKs is closely regulated throughout the various phases by different mechanisms.[80–82] CDK7 and CDK20 play a crucial role in transcription and cell cycle control. For therapeutic drugs, the suppression of CDKs via transcription and regulators of the cell cycle is regarded as a good strategy.[83–89]

Deregulation of CDK activity has also been detected in other cancers. Various studies have been performed to identify small molecules that target the CDKs, and numerous clinical trials have been conducted with pan-CDKIs.[90]

CURRENT DRUGS FOR HCC

The development of HCC involves dysregulation of the cell cycle, apoptosis, and many other cellular pathways. Tumor cell progression involves mutations in various proteins responsible for the regulation of cell cycle.[80] Hence, the recent advances in the treatment of HCC include molecules that target proteins such as CDKs or growth factors to suppress the tumor development.[91]

Currently approved drugs

Sorafenib

The first orally administered drug approved to target multiple kinases was Bayer's Nexavar (sorafenib), and it is currently the standard of care for systemic treatment of patients with advanced HCC who are not potential for curative options such as surgical resection, liver transplantation, and pharmaceutical interventions. Sorafenib inhibits PDGF-α and PDGF-β, VEGFR-1, VEGFR-2, and VEGFR-3, c-kit, and various proteins of the kinase cascade, Ras, C-Raf, B-Raf, ERK, as well as MAPK.[91–93] Recent studies have shown that inhibition of this kinase cascade by sorafenib has the ability to correct abnormal glycosylation in HCC cells by a reduction in expression of the protein Ets-1, which provided a significant improvement in the overall survival (OS).[94] In spite of the proven efficacy of sorafenib to significantly increase the OS in patients with advanced HCC, it was unable to stop the disease progression because of development of resistance to antiproliferative therapies.[95,96] It also failed to be an economical treatment method for patients with advanced HCC, and there is no ultimate treatment for second-line therapy in patients who fail to respond to sorafenib treatment.

Regorafenib

Regorafenib (Stivarga) developed by Bayer received approval in June 2017 as a second-line oral drug for unresectable HCC.[97] It has similar targets and structure as sorafenib but is a more effective inhibitor of STAT3 signaling. It does so by inducing src homology 2 domain–containing phosphatase 1 (SHP1).[98] In addition, it inhibits various oncogenic factors such as V600-mutated BRAF and Tie2.[97] Sorafenib-refractory patients who received regorafenib as a second-line therapy were shown to provide a survival benefit. Furthermore, regorafenib has shown more potency in inhibiting TKs and phosphatases than sorafenib and a better drug tolerance profile in patients with HCC.[99] In patients treated with regorafenib, the median survival was observed to be 10.6 months as compared to 7.8 months in placebo. As regorafenib offers this survival benefit in patients with HCC, it can be considered in combination with other drugs in patients where regorafenib cannot be tolerated in high doses or after sorafenib. At present, regorafenib is the standard second-line chemotherapy for patients refractory to sorafenib. However, only a few patients were eligible for regorafenib treatment due to their intolerance to sorafenib and deterioration of liver function.

Nivolumab

Human anti–programmed cell death protein 1 (PD1) (nivolumab) was approved by the FDA for advanced HCC after the molecular targeted therapy with sorafenib. Anti-PD1, with the brand name Opdivo, was developed by Bristol-Myers Squibb to prevent the immune tolerance in tumors. This human monoclonal antibody prevents the association of PD1 with its ligands, programmed death ligand1 (PD-L1) and 2 (PD-L2) as this binding otherwise leads to a poor T-cell response toward HCC tumors.[100] Nivolumab is anticipated to be a promising immune checkpoint inhibitor to treat HCC with a good safety profile.[101] New strategies such as combining sunitinib with other immunotherapeutic drugs such as anti-PD1 to inhibit tumor growth and to develop more economical combination therapies are currently in consideration. In this direction, Opdivo from Bristol-Myers Squibb Company is indicated for the treatment of patients with HCC. However, treatment with Opdivo resulted in some serious adverse reactions such as fatigue, abdominal pain, rash,

cough, and decreased appetite in patients with HCC. Hence, Opdivo has been discontinued due to adverse reactions in 11% of patients, and 32% of patients had a dose delay due to an adverse reaction.[101]

Lenvatinib

Lenvatinib (Lenvima by Eisai) is more effective than sorafenib in targeting angiogenesis in hepatocarcinoma cell lines.[102] Lenvatinib has also proven to be superior to sorafenib in increasing the OS in patients with HCC where tumors cannot be surgically removed. It targets VEGFA, VEGFC, fibroblast growth factor (FGF-1-4), and stem cell factor (SCF) to reduce angiogenesis and lymphoangiogenesis.[102] Its higher potency than sorafenib in targeting FGF-1– and FGF-2–orchestrated angiogenesis makes it a promising candidate for HCC.[102] Other targets of lenvatinib include VEGFR3, KIT, RET and PDGF receptor α.[103] To overcome the problem of acquired resistance to lenvatinib, it is being tested in combination with golvatinib.[104] The half inhibitory concentrations of different inhibitors on tumor cell lines, as published in the literature, are listed in table 1.

Cabozantinib

Cabozantinib (Cabometyx by Exelixis), an orally bioavailable multikinase inhibitor targeting MET, RET, VEGFR, and AXL, was approved by the European Commission in patients with HCC who have been previously treated with sorafenib. The approval is based on the results from a phase III CELESTIAL trial that showed significant improvement in OS in patients with advanced HCC. It has also been approved by the FDA for the treatment of advanced renal cell carcinoma and medullary thyroid cancer. This dual VEGFR/MET blockade is important as the therapies that are resistant to VEGFR are considered to be arising from the upregulation of proangiogenic pathways including the MET pathway.[114] Moreover, results from a subgroup analysis of patients with advanced HCC who received sorafenib showed that the median OS was 11.3 months versus 7.2 months for placebo and median progressive-free survival (PFS) was 5.5 months versus 1.9 months with placebo. However, the noted adverse effects were fatigue (12.4%), diarrhea (17%), and palmar-plantar erythrodysesthesia (8.7%).[115]

Table 1 IC_{50} Values of Drug in Different HCC Cell Lines.

Drugs for hepatocellular carcinoma	HCC cell lines	IC_{50}
Sorafenib[105–109]	HepG2 MHCC97H HepG2.215	8–9.9 μM 12–31 μM 5–7 μM
Sunitinib[110,111]	HepG2	32–49 μM
Lenvatinib[112]	HepG2	0.25 μM
Regorafenib[113]	HepG2 Hep3B	1 μM 5 μM

HCC, hepatocellular carcinoma.

Pembrolizumab

Pembrolizumab (Keytruda) developed by Merck is a recombinant humanized IgG4 monoclonal antibody against PD1, which was approved by the FDA for patients with advanced HCC pretreated with sorafenib. The FDA approval was based on findings from a phase II KEYNOTE-224 trial of 104 patients with pretreated liver cancer who received pembrolizumab. It has also been approved by the FDA in 2014 to treat patients with advanced melanoma and non–small-cell lung cancer who do not respond to other treatments. The most common treatment-related adverse events are fatigue, increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and hypothyroidism. Results from phase II KEYNOTE-224 trial showed a median PFS of 4.9 months and OS of 12.9 months.[116] A list of currently approved drugs by the FDA for the treatment of HCC is given in table 2.

Combination therapy

Treating patients with HCC has become very challenging because of the heterogeneity in the patient population

Table 2 Currently Approved Drugs for HCC.

Drugs	Developed by	Target	Therapeutic line	Trial	Approved for HCC (Year)
Sorafenib (Nexavar)	Bayer and Onyx	Multitargeted TKI	1	SHARP	2007 in the US
Regorafenib (Stivarga)	Bayer	Multitargeted TKI	2	RESORCE	2017 in the US
Nivolumab (Opdivo)	Bristol-Myers Squibb	PD1 immune checkpoint inhibitor	2	CheckMate-040	2017 in the US
Lenvatinib (Lenvima)	Eisai Co.	Multitargeted TKI	1	REFLECT	2018 in the US
Cabozantinib (Cabometyx)	Exelixis Inc.	Multitargeted TKI	2	CELESTIAL	2018 in Europe
Pembrolizumab (Keytruda)	Merck	PD1 immune checkpoint inhibitor	2	KEYNOTE-224	2018 in the US

HCC, hepatocellular carcinoma; PD1, programmed cell death protein 1; TKI, tyrosine kinase inhibitor.

and the variation in the risk factors involved in each patient. Moreover, many molecular pathways are involved in the development of drug resistance in HCC cells. Therefore, understanding these molecular mechanisms and combining drugs with other molecular or immune therapies to overcome these drawbacks has become an area of utmost importance.[117] Many studies were conducted to understand the characteristics of sorafenib-resistant HCC cells and to determine the mechanisms responsible for acquired resistance to sorafenib. It was observed that the resistant HCC cells highly expressed activated STAT3, JAK1, JAK2, AKT, and p85.[95] Recently, miRNAs highly expressed in HCC have been found to be good therapeutic targets to overcome resistance to sorafenib. One of the strategies was to suppress miR-222/221 as it induces sorafenib resistance via activation of the PI3K/AKT pathway.[118] Moreover, miR-181a plays a major role in inducing sorafenib resistance by inhibiting the protein RASSF1.[119] It has also been shown that downregulation of proapoptotic factors such as BMF, BIM, PUMA and PTEN by miR-221 promotes drug resistance and tumor survival.[120] Interestingly, miR-122 targets the IGF-1 and can be used in combination with sorafenib to overcome the problem of acquired drug resistance.[121] In some in vivo studies, when sorafenib was combined with vinorelbine, there was no increase in the toxicity levels or any reduction in the efficacy, but it was accompanied by slowing down the tumor growth compared with the individual monotherapies.[122] In phase III clinical studies, comparing sorafenib to placebo in differentiated thyroid cancer, the median PFS time was 10.8 months in the sorafenib group compared to 5.8 months in the placebo group.[123] Studies are going on for sorafenib in combination with transarterial chemoembolization (TACE) for the treatment of HCC as chemoembolization triggers are associated with metastasis and the spread of cancer cells around the body. Hence, using sorafenib with the antiangiogenic mechanism of action could potentiate the effects of TACE.[110] In another phase II study, the efficacy of sorafenib–gemcitabine was evaluated in patients with advanced HCC, and it was considered to be an unsafe therapy as gemcitabine-related thrombocytopenia was followed by sorafenib-related hand-foot skin reaction and anorexia was observed in patients with this combination therapy.[124] Because of these findings, there is an urgent need in clinical trials for sorafenib to be used in combination with different anticancer drug candidates.

Tiziana Lifesciences has recently licensed out a new form of CDK inhibitor called as Milciclib. Milciclib maleate is in phase II clinical development for the treatment of patients with cancer and is identified as a potent CDK2 inhibitor and has activity toward closely related CDKs, that is CDK1, CDK4, and CDK5 and tropomyosin receptor kinase A (TRKA). The postulated mechanism of action of the compound, as determined in biochemical assays, is that it blocks the G1 phase of the cell cycle. Milciclib has shown to lower tumor growth through downregulation of miR-221 and miR-222 in an animal model of HCC. Phase I clinical studies of Milciclib have shown improvement in patients with advanced solid tumors of colon, thymus, and pancreas. Therefore, Milciclib can be a potential candidate alone or in combination for the treatment of patients with HCC. In combination studies, Milciclib exhibited synergistic or more than additive activity when administered with gemcitabine.[125] In clinical phase II, this combination regimen has shown a promising advantage in about 36% of patients which also includes gemcitabine-resistant patients.[125] Also, Milciclib (a CDK inhibitor) has shown to reduce the expression of miR-221 and miR-222 in many in vitro models and can be tested in combination with sorafenib to eliminate the problem of miRNA-induced sorafenib resistance in HCC tumors, as described in Figure 1. Hence, Milciclib seems to be a promising candidate for combination therapies in patients with cancer.

To overcome the problem of acquired resistance to lenvatinib, it is being tested in combination with golvatinib.[104] Golvatinib (developed by Eisai) is an inhibitor of c-MET, vegfr-2, c-KIT, RON, Tie2, and EphB4. EphB4 and angiopoietin 2–activated Tie2 are responsible for acquired resistance against lenvatinib, and hence, lenvatinib was combined with golvatinib in preclinical studies to produce more effective results in HCC.[104] Moreover, regorafenib is being tested in combination with erlotinib, which inhibits platelet-derived EGFR signaling to increase its potency to inhibit proliferation of HCC cell lines. This combination was proposed as platelets have the ability to reverse the antitumor effects of regorafenib and sorafenib in vitro.[126]

Pfizer's palbociclib (PD0332991, ibrance) is a specific CDK4/6 inhibitor that induces the tumor suppressor AMP-activated protein kinase (AMPK) to downregulate the expression of protein phosphatase 5. This mechanism promotes apoptosis and autophagy in HCC cells.[127] Palbociclib arrests the cell cycle irreversibly by inhibiting phosphorylation of retinoblastoma (RB1) by CDK4/6, and the treatment also results in accumulation of cyclin D1. RB1 needs to be in its intact form for palbociclib to induce senescence of cancer cells in patients with HCC, and a mutation in the gene encoding this protein can lead to the development of resistance to this drug.[128] Palbociclib is being considered in a combination regimen with other drugs such as sorafenib to reduce adverse events and increase the OS in patients with HCC.[128] The schematic representation of combination therapy is displayed in Figure 1.

Owing to the late diagnosis and patient heterogeneity involved in HCC, therapies such as neoadjuvant or locoregional therapies have been widely used for tumor growth inhibition in patients with HCC. These therapies refer to

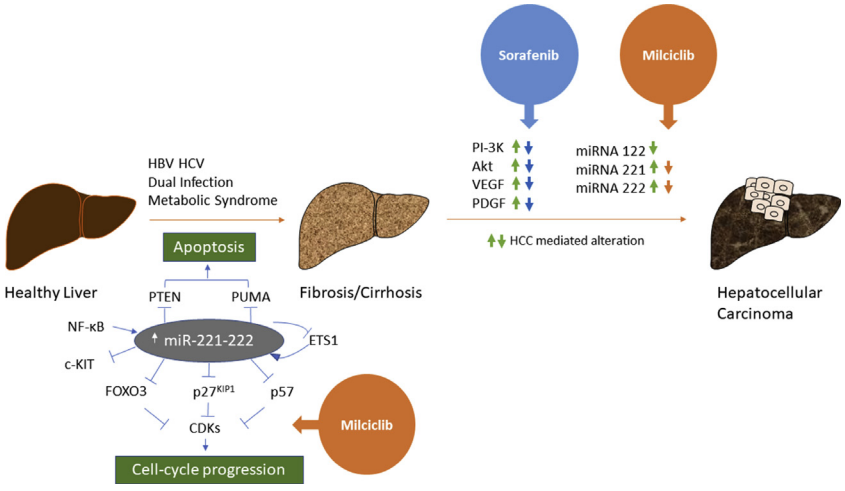

Figure 1 Schematic representation of combination therapy for HCC. The figure shows the factors that are responsible for the progression from a healthy liver to fibrosis and eventually hepatocellular carcinoma. Individuals with HBV and HCV infection and metabolic syndromes such as obesity or diabetes have a higher incidence of HCC. The most important players in HCC progression are miRNA 221 and 222 that inhibit major tumor suppressors such as PTEN, CDKN1B/p27^{kip}, CDKN1C/p57^{kip}, and PUMA. PTEN and PUMA induce apoptosis, whereas p27 and p57 keep a check on the CDKs which otherwise lead to uncontrolled cell cycle progression. The TKI sorafenib targets PI3K, AKT, VEGF, and PDGF but is unable to inhibit the miRNAs which lead to sorafenib resistance in patients with HCC. Hence, we need a drug that can overcome sorafenib resistance, and this can be achieved by combining sorafenib with milciclib as it downregulates miRNA 221 and 222. This combination therapy will improve the OS as it will take care of a multitude of antiapoptotic and carcinogenic factors. HCC, hepatocellular carcinoma; OS, overall survival; HBV, hepatitis B virus; HCV, hepatitis C virus; TKI, tyrosine kinase inhibitor.

a variety of treatment including systemic chemotherapy, TACE, radiofrequency ablation (RFA), hepatic resection, and transarterial radioembolization (TARE) that decrease the risk of dropout and improve the OS of patients with HCC.[129,130] However, various studies to examine the role of adjuvant or neoadjuvant or locoregional therapy alone in HCC are still under active investigation, but none of them have provided strong evidence in patients with HCC. Hence, treatment strategies continue to make progress to obtain better survival in patients with HCC. In this direction, the combination of molecular targeted therapies with locoregional therapies may have a beneficial effect on patient prognosis. Other than having promising results with TACE, sorafenib is also being evaluated in the neoadjuvant setting to prevent tumor progression and the risk of dropout of patients undergoing surgical resection and transplant. Another clinical trial of nivolumab in combination with ipilimumab as neoadjuvant therapy is ongoing beginning from April 2018. Hence, future trials will able to address the synergistic effect of combining molecular targeted therapies with neoadjuvant or locoregional therapy in patients with intermediate-HCC.

Drug candidates in the pipeline

Multiple companies and academic institutions have drugs under development for the treatment of metastatic HCC. Tiziana Lifesciences plc. has obtained Milciclib as a potent CKD2 inhibitor that belongs to the pyrazolo (4,3-h) quinazoline chemical class. It shows activity also toward closely related CDKs such as CDK1, CDK4, and CDK5 and TRKA. This profile has advantages over other CDKs as it may have the potential for synergistic inhibition. Milciclib possesses an unusual kinase inhibitory profile, as well as potent activity against a limited number of other kinases, such as members of the Src tyrosine kinase and splicing kinase families. It is in phase II clinical development as an oral anticancer treatment for patients with advanced hepatic malignancies. Also, Milciclib was found to be safe in patients with thymic cancers and thymoma.

Eli Lilly and company has galunisertib under phase II clinical trials for the treatment of HCC. It is a small molecule inhibitor that blocks TGF-β signaling.[131–133] Galunisertib may hinder cancer-initiating stem cells and thus prevent TGF-β–dependent tumor cell growth and migration.[134–136]

Sunitinib (Pfizer's Sutent), such as sorafenib, is an orally administered TKI but failed to be superior to sorafenib in clinical trials because of severe adverse events.[93,96] It targets tumor angiogenesis via VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, c-KIT, FMS-like tyrosine kinase 3, and other kinases.[93] Failure of sunitinib to treat advanced HCC may be contributed to its inability to reach the target micrometastases and islands of small tumors supported by the surrounding liver cells.[137]

Ipafricept is a potent antagonist of Wnt signaling, developed by OncoMed Pharmaceuticals and is currently under phase I clinical trials. Wnt signaling is implicated in strong antitumor activity in hepatocellular, breast, colorectal, and

other cancers.[138] Ipafricept is a recombinant fusion protein that includes extracellular ligand-binding domain of the human frizzled (FZD) 8 receptor and a human IgG1 Fc fragment.[139] It promotes differentiation, inhibits metastatic growth, and reduces cancer stem cell frequency. Ipafricept works either as a single agent or in combination with other approved drugs in patient-derived cancer xenograft models.[139] Bristol-Myers Squibb has developed Brivanib for the treatment of hepatocellular carcinoma, and currently, it is under phase III clinical trials. It is a selective inhibitor of fibroblast growth factor receptor and VEGFR and a type of antiangiogenesis agent.[140]

Ramucirumab (Cyramza, Lilly) is a recombinant IgG1 monoclonal antibody that antagonizes the VEGFR-2 activation and inhibits endothelial proliferation and migration. Recent reports suggested a survival benefit in patients with elevated alpha-fetoprotein (AFP) > 400 ng/ml at diagnosis and who progressed or were intolerant to sorafenib.[141] In a phase III clinical study comparing ramucirumab to placebo in advanced HCC, the objective response rate (ORR) of 9.5%, a median PFS of 4.0 months, and a median OS of 12.0 months were reported.[142] The most common adverse events were hypertension, hyponatremia, peripheral edema, and headache. It is in an ongoing phase III REACH-2 trial as a single agent in the second-line treatment (post-sorafenib) for patients with unresectable HCC and gastric cancer with elevated alpha-fetoprotein (AFP) levels. A list of future drugs in development for the treatment of HCC is given in table 3.

PROSPECTS FOR FUTURE RESEARCH

Molecular studies of HCC have determined abnormal activation of different signaling pathways, which illustrate key targets for novel molecular therapies. Other agents such as linifanib, ramucirumab, bevacizumab, axitinib, cediranib, dovitinib, vandetanib, oratinib, nintedanib etc. have demonstrated potential results in clinical phase 1-2 trials, but further studies are required to indicate their efficacy. Overall, combination therapies that would provide a synergistic effect and reduce drug toxicity are new directions for the upcoming treatments of HCC.

Table 3 Future Drugs for HCC.

Drugs	Developed by	Target	Phase
Milciclib (PHA-848125)	Tiziana LifeSciences	CDK2/TRKA	II
Palbociclib (Ibrance)	Pfizer	CDK4/CDK6	II
Galunisertib (LY2157299)	Eli Lilly	TGF-beta	II
Ipafricept (OMP-54F28)	OncoMed	Wnt signaling	I
Ipilimumab (Yervoy)	Bristol-Myers Squibb	CTLA-4 checkpoint inhibitor	II
Ramucirumab (Cyramza)	Eli Lilly	Anti-VEGF	III

TGF, transforming growth factor; CDK, cyclin-dependent kinase; TRKA, tropomyosin receptor kinase A; HCC, hepatocellular carcinoma.

CONCLUSION

In conclusion, genetic alteration leads to hepatocarcinogenesis that affects multiple signaling cascades and results in uncontrolled growth of the hepatocytes. There are systemic targeted therapies that focus on the critical steps of the carcinogenic pathways but limits in the widespread systemic toxicity. Hence, drugs such as Milciclib seem to be a promising candidate for combination therapies in patients with cancer. On account of the heterogeneity of HCC, proper combinative targeted therapy may improve the prognosis of advanced HCC. Therefore, combination therapy is an encouraging treatment modality for HCC.

CONFLICTS OF INTEREST

All authors have none to declare.

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