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Potential Molecular Targets and Drugs for Treatment of Hepatocellular Carcinoma

Abraham Nigussie Mekuria* and Abraham Degaga Abdi

College of Health Sciences, Addis Ababa University, Addis Ababa, Ethopia

Abstract

Hepatocellular Carcinoma (HCC) is a common type of primary liver cancer. According to the recent world cancer report, the disease ranked as the sixth most common cancer worldwide and the third largest cause of cancer-related death. Deregulation of numerous signaling pathways have been implicate in pathogenesis of HCC, including IGF, EGF/TGF, HGF/cMet, WNT, Hedgehog, notch, hippo, VEGF, PDGF, and FGF. Besides, intracellular mediators such as MAPK and PI3K/AKT/mTOR play a role in HCC development and progression. Currently sorafenib is the only molecularly targeted drug available to treat advanced HCC. It only extends survival by a matter of months. Moreover, there is no alternative agent for patients progressing under treatment with sorafenib. Thus, there remains a critical need for both continued molecular characterization and aggressive drug development. This review provided and updated appraisal of the deregulated signaling and epigenetic pathways, targeted therapeutics that is being investigated and possible challenges in drug development for HCC.

Keywords: Hepatocellular carcinoma; Signaling pathways; Epigenetic pathways; Targeted therapy

Introduction

Hepatocellular carcinoma (HCC) is a type of primary liver cancer that arises from hepatocytes, accounting for about 80% of primary liver cancer cases [1]. A total of 854,000 new cases of liver cancer and 810,000 related deaths were estimated in 2015. Globally, HBV accounted for 265000 liver cancer deaths (33%), alcohol for 245000 (30%), HCV for 167000 (21%), and other causes for 133000 (16%) deaths, with substantial variation between countries in the underlying etiologies [2]. For instance, in Africa and Asia, it is mainly due to endemic hepatitis B virus (HBV) infection, while hepatitis C virus (HCV), alcohol, non-alcoholic fatty liver disease are increasingly predominant causes in Western countries and Japan [3]. Moreover, in HBV endemic areas, there is usually an association with aflatoxin exposure which has a synergistic effect in increasing the risk of HCC development [4].

Literature Review

The long-term prognosis for HCC remains quite poor, with less than 12% of patients surviving 5 years from diagnosis [5]. Likewise, a recent study indicated that overall median survival of untreated patients with HCC is about 9 months [6]. The poor prognosis is thought to be a result of several factors. First, the disease is often very advanced at the time of diagnosis, as a result few patients eligible for curative surgical resection or liver transplantation. In addition, patients also tend to have little hepatic reserve function due to underlying liver disease and inherent resistance to systemic therapy [7].

Deregulated Signaling Pathways

Alterations of several signaling pathways have been implicated in the pathogenesis of HCC. Such as, pathways related to proliferation and survival, i.e. epidermal growth factor/transforming growth factor alpha (EGF/TGF-α), insulin-like growth factor (IGF), hepatocyte growth factor/cellular mesenchymal-epithelial transition (HGF/c-Met), and their intracellular mediators such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) have been deregulated in the disease. Besides, differentiation and development pathways, including wingless-type mouse mammary tumor virus integration site family member (wnt), hedgehog (Hh), notch, and hippo pathways as well as growth factor-regulated angiogenic signaling pathways like vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) were identified as a player in the imitation and progression of HCC (Figure 1) [8-12].

Tyrosine kinase receptor-dependent pathways

Growth factors which are linked to tyrosine kinase-receptor (TKR) have been known to trigger aberrant signaling related to cell proliferation, survival, and angiogenesis. This includes signaling pathways that involve EGF, TGF-α, IGF, HGF/c-Met, VEGF, FGF, PDGF and their intracellular mediator MAPK, and PI3K/AKT/mTOR [11,12].

EGF/TGFα pathway

Both EGF and the closely related molecule TGF-α bind to and activate EGF receptor (EGFR) [13]. Activation of the pathway stimulates cellular division, survival, and apoptosis [8]. Thus, any alteration in these pathways aids tumor growth and progression. Dysregulation of the pathway comes about thru several mechanisms in HCC, but largely because of overproduction of the ligand and studies reported that EGF over-expression is common in chronic hepatitis, cirrhosis, and HCC (40% to 80%) [14]. In support of this, increased EGFR signaling is a poor prognostic factor and associated with rapid tumor growth and increased the probability of intrahepatic metastases [15]. Additionally, over-expression of TGFα has been reported in pre-neoplastic lesions, suggestive of its role in early HCC [16]. Therefore, EGF/TGFα pathway

***Corresponding author:** Abraham Nigussie Mekuria, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia, Tel: 251911194519; E-mail: abrishn@yahoo.com

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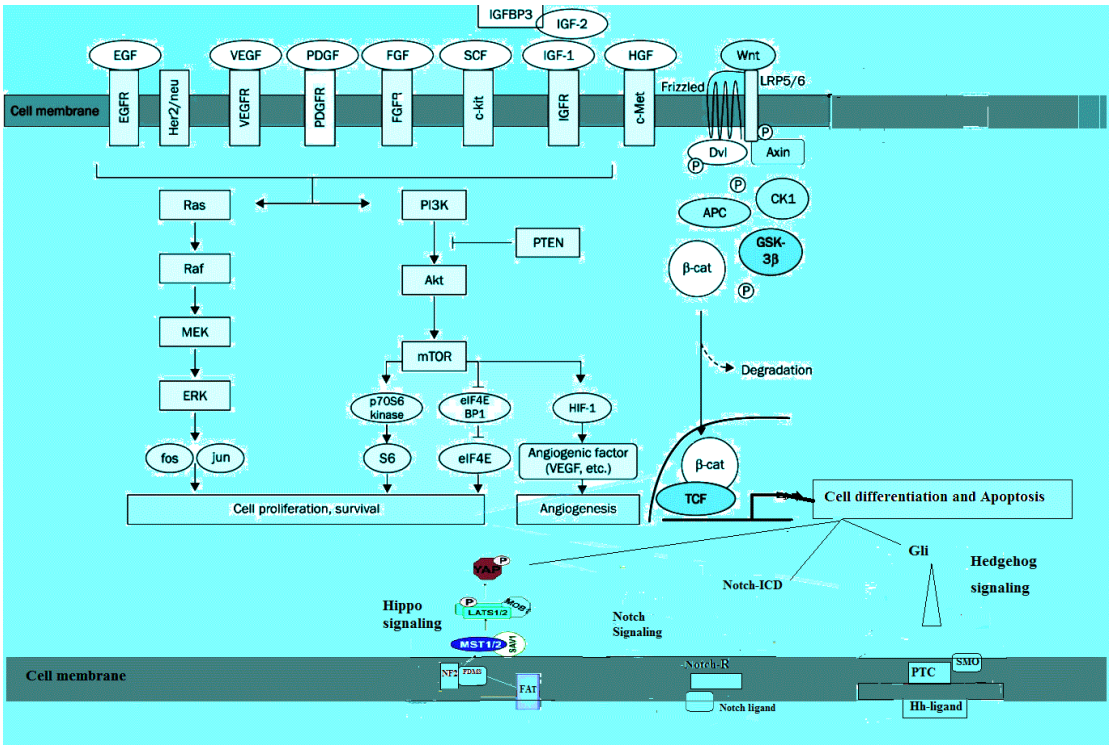

EGF: Epidermal Growth Factor; VEGF: Vascular Endothelial Growth Factor; PDGF: Platelet-Derived Growth Factor; FGF: Fibroblast Growth Factor; SCF: Stem Cell Factor; IGF: Insulin-Like Growth Factor; IGFBP: Insulin-Like Growth Factor Binding Protein; HGF: Hepatocyte Growth Factor; EGFR: EGF Receptor; VEGFR: VEGF Receptor; PDGFR: PDGF Receptor; FGFR: FGF Receptor; IGFR: IGF Receptor; MEK: Mitogen-Activated Protein Kinase/Extracellular Signaling-Regulated Kinase; ERK: Extracellular Signaling-Regulated Kinase; PI3K: Phosphoinositide 3-Kinase; PTEN: Phosphatase and Tensin Homolog; mTOR: Mammalian Target of Rapamycin; HIF: Hypoxia-Inducible Factor; LRP5/6: Low-Density Lipoprotein-Related Protein 5/6; GSK-3β: Glycogen Synthase Kinase 3β; CK1: Casein Kinase 1; β-cat: β-Catenin; APC: Adenomatous Polyposis coli; TCF: T-Cell Factor; Dvl: Disheveled; PTCH1: Patched1; SMO: Smoothened; GLI: Gliom Associated Oncogene; Nf2: Neurofibromin 2; Mst1/2: Mammalian Sterile 20-like Kinases 1/2; Mob: MEK Partner One Binder Protein; LAT: Large Tumor Suppressor; YAP: Yes Associated Protein; SAV: Salvador [8-12].

Figure 1: Potential molecular targets and intracellular signaling pathways in hepatocellular carcinoma.

has become a potential investigating area of research to identify target(s) to inhibit proliferation of HCC and metastasis.

IGF pathway

The pathway consists of circulating ligands IGF-I, IGF-II, and the receptor IGF-IR. It has been known for its involvement in the regulation of cell growth and energy metabolism [17]. According to studies, overexpression of IGF-II and IGF-IR has been implicated in cell proliferation and inhibition of apoptosis [18]. In support of this, low expression of IGF I and progressive increase in the level of expression of IGF-IR, IGF-II, and IGF substrates during the hepatocarcinogenesis process was detected at mRNA and protein level, which was associated with cell increased proliferation and reduced apoptosis has been observed in HCC cell lines and rat models as well as in human HCC samples [18]. Other studies also indicated that the major tumor-promoting effects of IGF ligands on HCC exerted through IGF-1R [19-22]. Knowing the involvement of IGF-1R pathway in the pathogenesis of HCC, researchers devised several strategies in therapeutic considerations in the treatment of HCC involving the pathway. The first method targets the ligand to reduce its activity, the second inhibit the function of the receptor, and the third modulates the downstream signal transduction [23].

HGF/c-Met pathway

HGF is a cytokine secreted by mesenchymal cells and c-Met is a receptor for the HGF. HGF/c-Met pathway plays a pivotal role in promoting cell proliferation, survival, and cell motility in a variety of neoplasms, including HCC [24]. HGF or Met over-expression, Met gene mutations and amplification inducing aberrant signaling have been reported in several cancers including HCC [25]. Even though c-Met overexpression being reported by the majority of studies no substantiation for a c-Met oncogenic 'addiction' exists in HCC. Nevertheless, c-Met over-expression was reported to be related to increased metastatic potential and poor prognosis in patients with HCC, providing a rationale for its therapeutic inhibition [26].

Pathways related to neo-angiogenesis

High vascularization is a hallmark of human HCC [8]. This angiogenesis process relies on autocrine and paracrine interactions between tumor cells, vascular endothelial cells, and pericytes. Tumor cells release pro-angiogenic factors in response to hypoxic conditions and nutrient deprivation and thus activate endothelial cells [27]. Activated endothelial cells break down extracellular matrix and basement membrane which result in a release of angiogenic factors which includes VEGF, FGF, PDGF, and TGF β [16]. These angiogenic factors in turn activate endothelial cells through TKR and their intracellular mediator's MAPK and PI3K/Akt/mTOR pathways, which lead to proliferation and migration of endothelial cells to form a new tubular structure and lumen for new vessels and finally, pericytes are activated and recruited to stabilize the new blood vessels [27,28].

Many studies have revealed that VEGF and FGFs as the most important stimuli for tumor angiogenesis in HCC [11,27]. Moreover, these studies also identified VEGF as the most potent angiogenic factor in HCC, and it is frequently found over-expressed in HCC tumor specimens, as well as its receptors VEGFR-1 and VEGFR-2 [29]. To inhibit angiogenesis, numerous studies have focused on targeting VEGF and VEGF receptor. Approximately one-third of molecularly directed therapies in a clinical evaluation are directed at VEGF or its receptor [30].

In addition to these pathways, angiopoetins (Ang) have also been involved in normal and aberrant vascular development through its interaction with the receptor Tie-2. Ang-2 has been designated as a promoter of tumor angiogenesis in the disease, particularly in the presence of VEGF [31]. In principle, targeted inhibition of angiogenesis can be achieved at different levels. These include the neutralization of growth factors with monoclonal antibodies, the inhibition of the downstream signaling from RTK, and the interference with the interaction between proliferating endothelial cells and matrix components [27].

MAPK pathways

MAPK is an intracellular signaling pathway downstream of several TKR such as EGFR, IGF-IR, PDGFR and VEGFR [32]. After ligand binding to the growth factor receptor, two downstream protein kinases are coupled to the receptor by Ras leading to its activation and subsequent activation of Raf serine/threonine kinases and MAP kinase kinase (MEK) activates extracellular signal-regulated kinase (ERK) via phosphorylation and the latter phosphorylates proteins involved in cell proliferation, inhibition of apoptosis, angiogenesis, and metastasis [30,33,34]. Based on, a study done by Huynh et al. [35], using 46 samples collected from HCC patients, overexpression of MEK1/2 (100% {46/46}) and ERK1/2 (91% {42/46}) as well as, ERK1/2 (69% {32/46}) phosphorylation were identified. The critical involvement of the MEK/ERK pathway in HCC tumorigenesis strongly suggests that the kinases MEK1/2 or ERK1/2 could be promising therapeutic targets.

PI3K/Akt/mTOR pathway

It is activated by signaling inputs transmitted to the inner cell after growth factor ligand binding to TKR such as EGFR or IGFR. Given its importance in cell growth and metabolism, it is not surprising that PI3K/AKT/mTOR plays a substantial role in HCC [36]. In a study done by Zhou et al. [37] using 528 HCC samples indicated that altered expression of pAKT, PTEN, p27 and S6 ribosomal protein (pS6), which was associated with poor survival. Moreover, PTEN mRNA expression in the cancerous tissue was down-regulated, compared with matched normal tissue. In another study done by Villanueva et al. [38] that aimed at analyzing 314 HCC samples for mutation detection, DNA copy number changes, determination of mRNA levels and protein by immunohistochemistry reported that PTEN the tumor suppressor that inhibits the mTOR pathway, is inactivated in around half of HCC tumors. Furthermore, the importance of mTOR in hepatocarcinogenesis has been shown in a mouse model with a liver-specific knockout of the negative regulator of mTOR, tuberous sclerosis complex 1 (Tsc1) resulted in chronic mTOR activation and led to the sporadic and sequential development of histological features associated with HCC [39]. Taken together, blockade of PI3K/AKT/mTOR signaling appears to be an attractive therapeutic strategy in HCC.

Signaling pathways related to cell differentiation and development

The ultimate identity of the specific target cell for transformation in HCC is still obscure. Despite recent progresses, the involvement of altered embryonic cellular features such as self-renewal, plasticity, asymmetric division, pluripotency and cellular fate in human cancer remains mysterious. Studies found dysregulation of pathways involved in cellular differentiation in HCC, such as the Wnt/*β-Catenin*, hedgehog, notch, and hippo signaling pathways [16].

Wnt pathway

The Wnt pathway is markedly decisive in the active surroundings of hepatic development and involved in the regulation of developmental processes such as differentiation, cell migration proliferation and survival of hepatocytes [40]. According to studies, aberrant signaling of the pathway can lead to a variety of human diseases ranging from birth defects to cancer [41].

Canonical Wnt pathway is the term used to describe a cascade that involves translocation of *β-catenin* from the cell membrane into the nucleus, where it acts as a coactivator of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors and in turn regulates specific target genes, including c-myc, cyclin D, and survivin [40]. In the absence of Wnt ligands, most cellular *β-catenin* associates with E-cadherin in adherence junctions at the plasma membrane. Cytosolic *β-catenin* associates in a complex with adenomatous polyposis coli (APC) and *AXIN1* or *AXIN2*, which mediates sequential phosphorylation of *β-catenin* by casein kinase 1 and glycogen synthase kinase 3 beta (GSK3 β). Phosphorylation of *β-catenin* triggers its ubiquitination by β-transducin repeat-containing protein and subsequent proteosomal degradation [22]. On the other hand, when the pathway is normally active, Wnt ligands form complexes with Frizzled (Fz) receptors and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) co-receptors. The formation of a likely Wnt-Fz-LRP6 complex together with the recruitment of the scaffolding protein Dishevelled (Dvl) results in LRP6 phosphorylation and activation and the recruitment of the Axin complex to the receptors. These events lead to inhibition of Axin-mediated *β-catenin* phosphorylation and thereby to the stabilization of *β-catenin*, which accumulates and travels to the nucleus to form complexes with TCF/LEF which regulates the transcription of important genes such as cyclin D, c-Myc, c-Met, FGF4, metalloproteinases, and VEGF. Gain-of-function mutations in the *β-catenin* gene and loss-of-function mutations in the *APC, AXIN1*, or *AXIN2* genes activate *β-catenin* signaling and oncogenesis [41].

Aberrant activation of Wnt/*β-Catenin* signaling has been reported in a wide range of HCC patients. This might be due to either activating mutations of the *β-catenin* gene (*CTNNB1*), or loss-of-function mutations in APC and AXIN genes or overexpression of frizzled receptors or inactivation of cadherin-1 [42]. Indeed, 40% to 70% of HCCs harbor nuclear accumulation of the *β-catenin* protein, one of the hallmarks of the pathway activation [43]. Moreover, HCC occurs in HCV patients, up to 40% of whom show an incidence of *CTNNB1* gene mutations [44]. Furthermore, studies of HCC occurring in patients with HBV have implicated protein X of the HBV to stimulate the activation of *β-catenin*, representing an independent *CTNNB1* gene mutation [45]. Lastly, evidence suggests that this pathway represents an important molecular target for HCC therapy.

Hedgehog pathway

Hedgehog (Hh) signaling pathway is crucial for embryogenesis and regulation of a variety of essential functions, from differentiation to regeneration, as well as in stem cell biology, through control of cellular proliferation, apoptosis, and migration [46]. In mammals, the Hh pathway is initiated by three related ligands, sonic hedgehog

(SHh), Indian hedgehog (IHh) and desert hedgehog (DHh). These ligands induce signaling by binding to Patched1 (PTCH1), inactivating PTCH1 and relieving inhibition of Smoothened (SMO), thus leading to the activation of gliom associated oncogene (GLI) transcription factors. But, unbound PTCH acts as a tumor suppressor that binds to and represses the proto-oncoprotein Smo. Thereby preventing it from activating downstream transcription factors, particularly the Gli1 [47]. Current studies linked Hh pathway in HCC pathogenesis [48]. Especially, activation of the SHh pathway appears to be imperative on both the development and the progression of HCC [49,50]. Chen et al. [50] reported a strong association between SHh pathway activation and tumor size, capsular invasion, as well as vascular invasion. This was supported by another study that identified SHh pathway induced cell migration and invasion through focal adhesion kinase/Akt signaling-mediated matrix metalloproteinase-2 and -9 production and activation in HCC [51]. Owing to its involvement in the tumorigenesis and progression of HCC, targeting proteins which are components of the pathway seems promising in the search for drugs against the disease.

Notch pathway

The pathway is highly conserved pathway that controls multiple cell differentiation processes during embryonic development and throughout adulthood [52]. In the liver, the pathway directs biliary fate and morphogenesis [53]. The pathway consists of Notch receptors, ligands, negative and positive modifiers, and transcription factors [54]. In mammals, the Notch system consists of four single pass transmembrane receptors (Notch1-4) and at least five membrane-anchored ligands (Jagged1, Jagged2, Delta-like (Dll)-1, 3, and 4). Notch receptors are constrained in a dormant state before ligand-induced activation that initiates a series of successive proteolytic cleavages. The final intramembrane cleavage is catalyzed by γ-secretase, a multisubunit protein complex, and leads to the release of Notch intracellular domain. This protein fragment then translocate into the nucleus and functions as a cofactor to regulate transcription of Notch target genes [55].

Deregulation in the pathway has been identified in many types of human cancers, including HCC, though its involvement in cancer development is complex, because Notch can function as an oncogene or a tumor suppressor depending on the tissue and on the presence of different signaling pathways [56,57]. Studies have revealed that Notch1 overexpression inhibits HCC cell growth by stimulating cell cycle arrest and apoptosis [57]. In contrast, other studies suggest an oncogenic role for Notch activation in the pathogenesis of HCC and showed the over activation of the signaling pathway detected in human HCC samples and also promotes formation of liver tumors in mice [58,59]. Therefore, Notch signaling pathway can be considered as one potential target for drug development.

Hippo pathway

It is an evolutionarily conserved pathway that controls organ size by regulating apoptosis, cell differentiation, and proliferation. In addition, dysregulation of the pathway contributes to cancer development [60]. Neurofibromin 2 (Nf2) is an upstream factor for the activation of the mammalian sterile 20-like kinases 1/2 (Mst1/2)–WW45 complex, which phosphorylates and activates Lats1/2 and the co-activator MEK partner one binder protein (Mob) 1A/1B. This activation of LATS inhibits the transcriptional co-activator Yes associated protein 1 (YAP1) and the co-activator with PDZ-binding motif tafazzin (TAZ) through their phosphorylation. Indeed, phosphorylated YAP1/TAZ cannot accumulate into the nucleus and this hinders their co-transcriptional activity [61]. Failure of the Hippo pathway leads to increased YAP1/TAZ activity with an under-phosphorylated form in the nucleus inducing oncogenic transformation due to the activation of transcription factors including transcription enhancer activation domain (TEAD). TEAD on its own is unable to induce gene expression and requires additional factors or co-activators for gene expression. Upon binding TEADs, YAP1/TAZ up-regulates the expression of several growth promoting factors, including connective tissue growth factor (CTGF), Cysteine-rich angiogenic inducer 61 (Cyr61), AXL RTK, c-Myc, survivin and amphiregulin (AREG) that is known to be an EGF family member [62].

Moreover, recent studies revealed that inactivation of the pathway could lead to inhibition of apoptosis, excessive cell proliferation, and subsequent carcinogenesis [63]. Furthermore, in these studies YAP1 has been implicated as an oncogene which has been altered in different kinds of human digestive system cancers, especially HCC [64]. For instance, a study aimed at evaluating the expression of YAP1 in 115 cases of human HCC samples has identified significant difference in YAP1 protein levels between normal and cancerous tissues [65]. Moreover, Su-xia et al. [66] investigated the expression of YAP1, TAZ, and AREG in HCC samples using immunohistochemical staining. The result indicated over expression of YAP1 in 69.2%, TAZ in 66.7%, and AREG in 61.5% of HCC patients. Furthermore, the study indicated that, expression of YAP1 was significantly correlated with stage, serum AFP level, and HCC prognosis. They suggested YAP1 maybe an independent prognostic indicator for HCC patients. On the other hand, YAP1 knockdown by siRNA-lipid nanoparticles dramatically restores hepatocyte differentiation in advanced HCC and leads to tumor regression [67]. These results suggest that, YAP1 activation plays an important role in HCC, and an impaired Hippo pathway might be a common mechanism for YAP1 activation. It is therefore believed that, the pathway can be deemed as a potential target for development of drugs in HCC.

Epigenetic Alterations

Epigenetic alterations are heritable alterations in the pattern of gene expression caused by mechanisms other than changes in the DNA sequence. This involves alterations in DNA methylation pattern, post translational modifications of histones and non-coding RNA expression profiles (especially microRNAs) [68]. The epigenetic regulation is a vital mechanism for cellular differentiation and cell fate decisions and is well-known to have significant role in HCC [69].

Alterations in DNA methylation pattern

DNA methylation in the mammalian genome is found at the cytosine residues of CpG dinucleotides, often associated with promoter related CpG islands [70]. The two most common forms of aberrant methylation are global hypomethylation and site-specific hypermethylation. While the former induces chromosomal and genomic instability, regional hypermethylation is usually related to the silencing of tumor suppressor genes [68]. For instance, aberrant DNA hypermethylation in promoter regions of tumor suppressor genes, such as cyclin-dependent kinase inhibitor 2A family of cell cycle inhibitors *(p16INK4A)*, *E-cadherin*, retrovirus associated sequence association domain family 1A (*RASSF1A*), suppressor of cytokine signaling (*SOSC-1*) and *PTEN* has been reported in HCC. According to studies, the frequency of aberrant DNA methylation increases from precancerous lesions to dysplastic nodules and finally HCC, indicating their importance in tumor progression [68]. As reported by Park et al. [71], cellular DNA methylation may be altered by HBV infection via DNA methyltransferases (DNMTs). Up regulation of *DNMT1*, *DNMT3A1* and *DNMT3A2* in cell cultures has been shown by hepatitis B virus X

Protein (HBx). These DNMTs mediate regional hypermethylation of tumor suppressor genes. The expression of *DNMT3B* is also suppressed by HBx that result in global hypomethylation of satellite 2 repeat sequences.

Alterations in post-translational modifications of histones

The posttranslational modification of histones, which package the DNA into chromatin, is an epigenetic change that affects chromatin condensation, DNA accessibility and transcriptional activity [69]. Histone (H) modifications comprise covalent posttranslational modifications of histone H proteins at the N-terminal domains of the core histones H2A, H2B, H3, H4, and the H1 family of linker histones via acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, biotynylation and others [68]. With respect to HCC, acetylation and methylation of H lysine residues are the best studied H modifications so far [68-70]. Accordingly, H acetylation is associated with an active transcription, whereas methylation may be associated with either active or repressive states, depending on the modified site [72]. Furthermore, studies have shown a number of transcriptionally repressed genes due to hypoacetylation of lysine residues at H3 or H4 [73]. Similarly, transcriptionally silenced retinoblastoma-interacting zinc-finger protein 1 *(RIZ1)*, *p16INK4A* and *RASSF1A* tumor-suppressor genes in human HCC were characterized by an increased level of repressive H3 lysine 9 and histone H3 lysine 27 methylation marks at their promoters [74].

Aberrant alterations in non-coding micro-ribonucleic acids (miRNAs)

The third epigenetic mechanism of gene regulation involves several non-coding miRNAs. They are single-stranded RNAs and serve as a post-transcriptional regulator of gene expression by interacting with mRNA [75]. In addition they participate in other activities including cell differentiation and development, cell proliferation and apoptosis, cell movements and stem cell renewal [76]. According to studies, the deregulation and dysfunction of miRNAs play a critical role in the pathogenesis of human cancers [77]. Moreover, numerous studies have linked aberrant expression of miRNAs with the initiation and progression of HCC [78,79]. Recently, El Hefnawi et al. [80] reported the result of an integrative meta-analysis study on the role of miRNA in HCC, and the report revealed positive contribution to HCC development is implicated for many down-regulated miRNAs that suppress important oncogenes, such as miR-122, miR-214, miR-199a-3p/5p and miR-34a, and for several up-regulated miRNAs that suppress tumor-suppressors, such as miR-182 and miR-186 oncomiRs. On the other hand, some miRNAs may play dual roles by targeting both tumor-suppressors and oncogenes. Therefore, without complete analysis of their targets and pathways, care should be taken in defining the role of deregulated miRNAs in liver cancer [80].

Drugs in the Pipeline

It is known that many molecular pathways have been well described in the development and progression of HCC. Thus, each of these molecular pathways provides an opportunity to develop agents that might slow down, halt or reverse the progression of HCC. In the recent years, a large number of new molecularly targeted agents have been investigated, including inhibitors of EGFR, IGFR, HGF/c-Met linked pathways, and angiogenesis inhibitors [10]. In addition, inhibitors of intracellular signaling pathways like MAPK, mTOR are also under investigation [81].

EGFR inhibitors

Currently two major classes of EGFR inhibitors have been developed some of these drugs are approved for cancer therapy. They are small molecule EGFR tyrosine kinase inhibitors (TKIs) including erlotinib, gefitinib, lapatinib, and imatinib and monoclonal antibodies against EGFR including cetuximab. Such anti-EGFR drugs have already been introduced in clinical studies in monotherapies and combined therapies [82].

Among the anti-EGFR drugs, erlotinib was considered as the most effective single-agent novel molecularly targeted therapy against HCC. This was based on the evidence from the results obtained in two randomized controlled phase II trials done by Philip et al. [83] and Thomas et al. [84] who examined the role of erlotinib in patients with advanced inoperable HCC. The trials enrolled a total of 78 patients and the average disease stabilization rate reached 51%, whereas average progression-free survival (PFR) and OS achieved durations of 3 and 12 months, respectively. In view of the modest antitumor activities of erlotinib, Zhu et al. [85] conducted multicenter, multinational, randomized phase III trial (Table 1) that aimed at comparing the clinical outcomes of sorafenib plus either erlotinib or placebo in patients with advanced HCC. The study enrolled 546 adult HCC patients who failed sorafenib treatment (during or after therapy) or who were ineligible for sorafenib treatment in the first place. Patients were randomized to everolimus plus best supportive care (BSC) and placebo plus BSC groups. But, as reported by the author's, no statistically significant differences in median TTP and OS were achieved among both treatment groups. In conclusion, to date except for the fairly moderate antitumor effects associated with erlotinib, the remaining drugs belonging to EGFR inhibitors have failed to demonstrate any substantial antitumor effects as monotherapy in patients with advanced HCC [59].

IGF inhibitors

IGF-targeting drugs are currently being developed and mainly including anti-IGF-1R antibodies, such as BIIB022, AVE1642 and cixutumumab (IMC-A12) [79]. Among anti-IGF-1R antibodies, cixutumumab exhibited anti proliferative, apoptotic, and reduction of tumor growth in preclinical studies; as well partial anticancer activity in phase I clinical trial [9]. On the other hand, a phase II clinical trial in 22 patients with advanced HCC had not demonstrated clinically meaningful antitumor activity of cixutumumab as monotherapy [86].

Anti-angiogenic agents

The hyper-vascular nature of HCC as evidenced by increased expressions of micro-vessel concentration and VEGF has led to increasing interest in exploring the potential of anti-angiogenic therapy in this disease [27]. Accordingly, numerous antiangiogenic drugs have been introduced in clinical studies in mono- and combined therapies. These drugs include bevacizumab, sunitinib brivanib, pazopanib, inifanib, cediranib, orantinib, ramucirumab, vatalanib, ramucirumab, regorafenib, axitinib linifanib, lenvatinib and others [9].

Sunitinib and linifanib, both primarily targeting VEGFR and PDGFR, failed to prolong OS compared to sorafenib (Table 1), and were associated with relatively more grade 3 or 4 adverse events than sorafenib [87,88]. On the other hand, brivanib, which targets VEGFR, PDGFR and FGFR, also failed to prolong OS (Table 1) in a phase III trial conducted to investigate its efficacy as a first line therapy even though it had a more favorable toxicity profile than sorafenib [89,90]. Moreover, another phase III, randomized, placebo-controlled study investigated the efficacy of brivanib after sorafenib failure and the authors reported that, in comparison to placebo, brivanib resulted in a longer median TTP but insignificant increase in the OS (Table 1) [91-108].

Agent/s	Target/s	Trial	OS; median (months)	PFS/TTP; median (months)	Ref.
Sorafenib	VEGFR	III (SHARP): sorafenib vs placebo	10.7 vs 7.9	5.5 vs 2.8	[107]
Sorafenib	VEGFR	III (Asia Pacific): sorafenib vs placebo	6.5 vs 4.2	2.8 vs 1.4	[108]
Sorafenib/ erlotinib	VEGFR/EGFR	III (SEARCH): Sorafenib/erlotinib vs sorafenib/placebo	8.5 vs 9.5	4.0 *vs* 3.2	[85]
Sunitinib	VEGFR/PDGFR/ c-KIT/FLT3	III: sunitinib vs sorafenib	7.9 vs 10.2	PFS; 3.6 vs 3.0	[87]
Brivanib	VEGFR/FGFR	III (BRISK-FL): brivanib vs sorafenib	9.5 vs 9.9	4.2 vs 4.1	[90]
Brivanib	VEGFR FGFR	III (BRISK-PS): brivanib vs placebo	9.4 vs 8.2	4.2 vs 2.7	[91]
Linifanib	VEGFR/PDGFR	III: linifanib vs. sorafenib	9.1 vs 9.8	5.4 vs 4.0	[88]
Ramucirumab	VEGFR-2	III (REACH): ramucirumab + BSC vs placebo + BSC	9.2 vs 7.6	1.48 vs 2.63	[92]
Lenvatinib	VEGFR//FGFR, RE/KIT/ PDGFR	III (REFLECT): lenvatinib vs sorafenib	Results pending	Results pending	[93]
Everolimus	mTOR	III (EVOLVE1): everolimus vs placebo	7.56 vs 7.33	2.96 vs 2.60	[99]

BSC: Best Supportive Care; OS: Overall Survival; PFS: Progression-Free Survival; TTP: Time to Progression; mTOR: Mammalian Target of Rapamycin; EGFR: Epidermal Growth Factor Receptor; PDGFR: Platelet Derived Growth Factor Receptor; FGFR: Fibroblast Growth Factor Receptor; BRISK-FL: Brivanib Study in Patients at Risk-First-Line; BRISK-PS: Brivanib Study in Patients at Risk-Post Sorafenib; SHARP: Sorafenib Hepatocarcinoma Assessment Randomized Protocol.

Table 1: Completed phase III studies with targeted therapies in advanced hepatocellular carcinoma.

Agent/s	Target/s	Trial	Current Status	NCI trial identifier
Erlotinib + Bevacizumab	EGFR/VEGFR	Phase II: single group assignment	Active, not recruiting	NCT01180959
Brivanib	VEGFR, EGFR	Phase III: brivanib + BSC *vs* Placebo + BSC	Active, not recruiting	NCT00825955
Lenvatinib	VEGFR, PDGFR, c-KIT, FLT3	Phase III: lenvatinib *vs* sorafenib	Active, not recruiting	NCT01761266
Ramucirumab	VEGFR-2	Phase III: ramucirumab *vs* Placebo	Recruiting	NCT02435433
Regorafenib	VEGFR2/ TIE2/ RAF/RET/c-KIT	Phase III: Regorafenib *vs.* placebo	Active, not recruiting	NCT01774344
Apatinib	EGFR	Phase III: apatinib *vs* Placebo	Recruiting	NCT02329860
Tivantinib	c-MET	Phase III: tivantinib *vs* Placebo	Active, not recruiting	NCT01755767
Cabozantinib	c-MET	Phase III: cabozantinib *vs* placebo	Recruiting	NCT01908426
Temsirolimus + sorafenib	mTOR/VEGFR	Phase II: single group assignment	Active, not recruiting	NCT01687673
Refametinib + sorafenib	MEK/VEGFR	Phase II: single group assignment	Active, not recruiting	NCT01915602
Mapatumumab + sorafenib	TRAIL-R1/VEGFR	Phase II: mapatumumab + sorafenib *vs* sorafenib + placebo	Active, not recruiting	NCT01258608
Axitinib	VEGFR/c-KIT/PDGFR	Phase II: single group assignment	Recruiting	NCT01273662
		Phase II: axitinib + BSC *vs* Placebo + BSC	Active, not recruiting	NCT01210495
		Phase II: single group assignment	Active, not recruiting	NCT01334112

c-MET: Cellular Mesenchymal Epithelial Transition; MEK: Mitogen-Activated Protein Extracellular Kinase; VEGFR: Vascular Endothelial Growth Factor Receptor; PDGFR: Platelet Derived Growth Factor Receptor; TRAIL-R1: TNF-Related Apoptosis-Inducing Ligand; mTOR: Mammalian Target of Rapamycin; EGFR: Epidermal Growth Factor Receptor.

Table 2: Ongoing phase II and III studies with targeted therapies in advanced hepatocellular carcinoma. (Last updated November 3, 2017).

Ramucirumab, a specific inhibitor of VEGFR-2, has shown positive result in phase I and II studies that prompted the initiation of the phase III REACH trial in HCC which compared ramucirumab/supportive care with placebo/supportive care for second-line treatment after sorafenib. While the REACH trial's primary endpoint of OS favored the ramucirumab arm, it was not statistically significant (Table 1) [92]. On the other hand, lenvatinib, an oral multi-targeted TKI of VEGFR, PDGFR, FLT3, and c-KIT, has shown highly promising response data in phase I/II clinical trials in HCC, although with some concerns regarding its toxicity profile [93]. Recently, a phase III trial comparing lenvatinib to sorafenib has been completed, and the results of this trial are awaiting [94].

HGF/C-Met inhibitors

Based on preclinical studies, application of c-Met inhibitors to c-Met positive cells have showed increased apoptosis, decreased proliferation and suppressed tumor growth, while c-Met reduced cells survived the inhibition treatment. This suggests that c-Met inhibition may be an effective therapy only for selected patients with strong c-Met expression [95]. Currently developed agents which target the pathway are small molecules and antibody based therapies. Further, small molecule c-Met inhibitors can be classified as selective inhibitors (includes tivantinib, capmatinib, tepotinib) which specifically target c-Met tyrosine kinase in an ATP-competitive or non-competitive manner, or non-selective inhibitors (includes cabozantinib, foretinib, golvatinib, crizotinib) which target other kinases in addition to c-Met [82].

On the other hand, blockade of the HGF/c-Met pathway can also be effected through anti-HGF neutralizing antibodies (includes rilotumumab, ficlatuzumab), which block only HGF-dependent c-Met activation, or anti-Met antibodies (includes ornartuzumab, onartuzumab, emibetuzumab). Generally, current data from c-Met inhibitors is promising, with phase III trials in progress for tivantinib and cabozantinib [9].

Tivantinib demonstrated anti-cancer activity in a wide range of tumor cell lines, as well as in xenograft models [24]. Concerning clinical trials in HCC, in phase I and II studies the drug has demonstrated promising antitumor activity in patients with HCC; both as monotherapy and in combination with sorafenib [95]. For instance, a randomized phase II trial in second-line HCC revealed improved OS of 7.2 versus 3.8 months in patients with Met-high tumors, as verified by immunohistochemistry in HCC patients treated with tivantinib versus placebo [96]. Based on the encouraging results of the randomized phase II trial, a randomized, double-blind, stratified, placebo-controlled phase III trial was initiated. This ongoing study aimed at investigating the efficacy of tivantinib monotherapy as second-line treatment in patients with advanced, pretreated, Met-high HCC (Table 2).

Cabozantinib is a non-selective oral multi-kinase inhibitor targeting c-Met, VEGFR2, KIT, RET, FLT3 and TIE-2 [24]. The drug prolonged survival in a Met-driven transgenic mouse model of HCC, and has revealed clinical activity in patients with advanced HCC participated in a phase II randomized discontinuation study. As reported by the author's, hand-foot syndrome (15%), diarrhea (9%), and thrombocytopaenia (9%), were the most frequent grade 3 and higher adverse effects related with cabozantinib [97]. Based on the encouraging data from the phase II study, a phase III randomized double-blind study is currently ongoing to compare the efficacy of cabozantinib against placebo as second-line treatment for advanced HCC patients who have previously received sorafenib (Table 2).

mTOR inhibitors

Several drugs targeting mTOR pathway have already been introduced in clinical studies in monotherapies and combined therapies. Examples of mTOR inhibitors include everolimus, sirolimus, and temsirolimus [9].

Among the drugs targeting the mTOR pathway, everolimus stands out as the most effective novel molecularly targeted monotherapy despite its modest antitumor activities. Dose-limiting adverse effects are common and include infection, diarrhea, elevated alanine aminotransferase, elevated total bilirubin, cardiac ischemia, and reactivation of HBV/HCV [98]. Considering the preliminary data that suggested everolimus could extend survival as second-line therapy for HCC after sorafenib, leading to an international phase III trial that was conducted by Zhu et al. [99]. But the results of this multicenter, randomized, double-blind, phase III trial showed little difference between treatment arms and placebo group in terms of OS and TTP (Table 1).

MAPK inhibitors

The clinical efficacy of MAPK pathway inhibitors, including PD-0325901, PD032590, pimasertib (AST03026), selumetinib (AZD6244), rafametinib (BAY 86-9766, RDEA119), TAK733, binimetinib, RO5126766, WX-554, RO4987655, GDC-0973, AZD8330, and trametinib (GSK1120212), have been evaluated in several cancers (including solid tumors such as HCC). But, from such evaluated MAPK inhibitors, only selumetinib and rafametinib have showed antitumor activity against HCC [100].

A phase II study of the selective MEK inhibitor selumetinib in a biomarker-unselected population of patients with advanced-stage HCC in the first-line setting did not detect a significant objective radiological tumor response. Essentially, pharmacodynamic studies showed that selumetinib was able to block MEK signaling by preventing phosphorylation of ERK and MEK [101]. On the other hand, refametinib is a potent non-ATP competitive inhibitor of MEK 1 and MEK 2 [102]. Preclinical studies have demonstrated its activity as a single agent and in combination with sorafenib. Furthermore, a single-arm phase II study in Asian patients with advanced-stage HCC detected a median TTP of 122 days and median OS of 290 days [103].

Epigenetic Modifying Therapies

Currently, epigenetic modifying drugs which target DNA methyltransferases and histone deacetylases (HDAC) enzymes are under development for HCC [68].

Deoxyribonucleic acid methylation (DNMT) inhibitors

Studies done on cell lines and pre-clinical mouse models revealed encouraging antitumor activities of DNA methyltransferase (DNMT) inhibitors. Such results may open new avenues for the intervention and management of HCC. For instance, Andersen et al. [104] showed that treatment with the DNMT inhibitor zebularine caused inhibition of proliferation coupled with increased apoptosis, whereas drug-resistant cell lines were associated with up regulation of oncogenic networks (e.g. **E2F1**, **Myc**, and **TNF**) driving liver cancer growth **in vitro** and in preclinical mouse models. Based on these findings, the researchers concluded that zebularine may only benefit a specific sub-population of HCC patients.

Histone deacetylase (HDAC) inhibitors

The efficacy of HDAC inhibitors in HCC has been studied in preclinical and clinical studies. In preclinical investigation, belinostat inhibited cell growth in a HCC cell line, whereas vorinostat sensitized HCC cells to acetylation of p53 and TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [69]. Moreover, in a multi-center phase I/II clinical trial, belinostat was found to stabilize inoperable advanced HCC [105]. Furthermore, Lin et al. [106] reported that HDAC inhibitors induced cell death may be accompanied with simultaneously activating tumor-progression genes. These studies indicated that a more in-depth understanding of epigenetic mechanisms is necessary to obtain further insights into the **in vivo** determinants of responses to epigenetic drugs.

Discussion

Challenges in drug development

The 2007 approval of sorafenib by FDA paved the way for testing a wide range of molecularly targeted drugs [107,108]. Though, none of these drugs have demonstrated survival benefits in patients with HCC. The reasons for the unsatisfactory phase III clinical trial results thought to be multifaceted. Among them incomplete understanding of the key molecular changes that lead to HCC development, broad range of liver dysfunction seen in HCC patients, liver toxicity, errors in trial design and marginal antitumor potency [109-111].

Many solid tumors showing strong "oncogene addiction", in which the proliferation and survival of cancer cells depends on a single oncogene and usually responsive to its inhibitor or antibodies; for example, gefitininib targeting EGFR in lung cancer. However, no such oncogene dependency has yet been shown in HCC. Genome sequencing of HCC patients have identified several driver genes [112]. It is therefore, developing a clearer picture of the most prominent and relevant molecular abnormalities is fundamental to developing effective therapeutic options, and should be a priority of those involved in basic and translational research.

Moreover, the variety of disease etiologies and broad range of liver dysfunction seen in HCC patients confound designing and interpreting results from standard phase I, II, and III clinical trials. In typical phase II trial designs, with response rate as the primary end point, it is difficult to identify the significant inter-patient variability that exists in HCC patients due to the heterogeneous nature of the disease [85]. In addition, specific phase II studies exploring potential liver-related toxicities of new agents are required in patients with cirrhosis and HCC before new agents should be tested in phase III randomized controlled trials [110]. Furthermore, liver cirrhosis that coexist with HCC may leads to portal hypertension may result in hypersplenism with platelet sequestration, thrombocytopenia, esophagogastric varices and GI bleeding, hepatic encephalopathy, hypotension, and hypoalbuminemia that may result in differential drug binding and altered pharmacokinetics [109].

It is generally accepted that targeted cancer drugs cannot be used on a 'one size fits all' model and trials need to reflect the original fundamental biology [112]. This means designing trials with solid pre-clinical laboratory work and where the patient subgroup to be treated is refined according to precise biomarkers, such as specific oncogene mutations. The emerging trials with tivantinib will provide the first indication that stratifying patients to specific treatment regimens will perhaps improve care [95,96,113].

Conclusion and Future Perspectives

Currently, the molecular profile of HCC is still too obscured compared with more common malignancies, such as breast, lung, and colon cancer, and this largely prevents investigators in designing and conducting molecularly oriented clinical trials. Additionally, it will be imperative to know in more details the inter-relations among different pathways; so as to design rationale drug combinations and treatment sequences. Furthermore, identifying clinical and biological factors, which may help selecting patients with higher chances of benefit, is essential in order to hasten drug development and maximize treatment efficacy.

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