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Review Article

Molecular genetics of hepatocellular neoplasia

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Abstract: Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third leading cause of cancer deaths worldwide. Proper classification and early identification of HCC and precursor lesions is essential to the successful treatment and survival of HCC patients. Recent molecular genetic, pathologic, and clinical data have led to the stratification of hepatic adenomas into three subgroups: those with mutant *TCF1/HNF1 α* gene, those with mutant β–catenin, and those without mutations in either of these loci. Hepatic adenomas with β-catenin mutations have a significantly greater risk for malignant transformation in comparison with the other two subgroups. Telangiectatic focal nodular hyperplasia has now been reclassified as telangiectatic adenoma due to the presence of non-random methylation patterns, consistent with the monoclonal origin which is similar to hepatic adenoma and HCC. HCC precursor lesions demonstrate unique molecular alterations of HSP70, CAP2, glypican 3, and glutamine synthetase that have proven useful in the histologic diagnosis of early HCC. Though specific genetic alterations depend on HCC etiology, the main proteins affected include cell membrane receptors (in particular tyrosine kinase receptors) as well as proteins involved in cell signaling (specifically Wnt/beta-catenin, Ras/Raf/MEK/ERK and PI3K/Akt/mTOR pathways), cell cycle regulation (i.e. p53, p16/INK4, cyclin/cdk complex), invasiveness (EMT, TGF-β) and DNA metabolism. Advances in gene expression profiling have provided new insights into the molecular genetics of HCC. HCCs can now be stratified into two clinically relevant groups: Class A, the low survival subclass (overall survival time 30.3± 8.02 months), shows strong expression signatures of cell proliferation and antiapoptosis genes (such as PNCA and cell cycle regulators CDK4, CCNB1, CCNA2, and CKS2) as well as genes involving ubiquitination and sumoylation; Class B, the high survival subclass (overall survival time 83.7 ±10.3 months), does not have the above expression signature. In fact, insights into HCC-specific alterations of signal transduction pathways and protein expression patterns have led to the development of new therapeutic agents with molecular targets such as EGFR, VEGF, or other multikinase inhibitors. In the future, these specific molecular alterations in HCC can potentially serve as diagnostic tools, prognostic markers, and/or therapeutic targets with the potential to alter clinical outcomes.

Key words: Molecular genetics, liver cancer, hepatocellular carcinoma, HSP70, CAP2, glypican 3, glutamine synthetase, β-catenin

Hepatocellular neoplasms mainly consist of hepatic adenoma, hepatocellular carcinoma (HCC) and precuror lesions. Benign tumors such as hepatic adenoma, while usually not deadly, may cause signfincant clinical challenges inlcuding maligant transformation. HCC due to vaious etiologies is one of the major leading causes of cancer death worldwide. Although knowledge about HCC is expanding exponentially in recent years, treatment and prevention of HCC is still a big challenge, and requires our thorough understanding of the molecular mechanisms of hepatocarcinogenesis. In this review article, we summarize the recent findings on molecular genetic pathology of hepatocellular neoplasms that have potential clinical implication in diagnosis, prognostication, and/or therapy.

Hepatic Adenoma

Hepatic adenoma predominantly occurs in younger women who are of child-bearing age, with or without prolonged use of oral contraception or abnormal carbohydrate metabolism (i.e. familial diabetes mellitus, glycogen storage disease, or galactosemia). It is usually a single nodule and sporadic, but can present as multiple tumors (adenomatosis) and have a familial inheritance pattern (familial liver adenomatosis).

The major clinical significance includes spontaneous hemorrhage, rupture, and, in rare instances, malignant transformation [1-3].

Molecular basis of pathogenesis

The molecular mechanisms by which an adenoma arises from hepatocytes are not well understood. However, comparative genomic hybridization (CGH) data [4] suggest associations between tumorigenesis and frequent Wnt/β-catenin activation (20-34%) [5, 6], bi-allelic loss of function mutations in the genes encoding hepatocyte nuclear factor 1 α (HNF1α) or TCF1 (50%) [7], allelic imbalances in chromosomal arms 11p, 13q and 17p [8], and gains of 1q. [9]

Molecular diagnosis and classification

While the diagnosis of hepatic adenoma can generally be made histologically, with or without radiological correlation, understanding the molecular genetics of hepatic adenomas may be clinically relevant. Based upon the mutation analysis of *TCF1/HNF1α* and *β-catenin*, a study proposed to sub-classify hepatic adenomas into three groups: 1) The most common and clinically important group has *HNF1α* mutation and is characterized by marked steatosis, lack of cytologic abnormalities, and no inflammatory infiltrate; 2) Tumors with only *β-catenin* activation have frequent cytologic abnormalities and pseudo-glandular formation; and 3) Tumors without *TCF1/HNF1α* or *β-catenin* activation mutation have frequent cytologic abnormalities, ductal reaction, and dystrophic vessels with/without inflammatory infiltrates[9]. Accordingly, compared to *TCF1/HNF1α* mutation, *β-catenin* activation is more frequently associated with a high risk of malignant transformation in adenomas [9].

Although telangiectatic focal nodular hyperplasia (TFNH) is morphologically similar to focal nodular hyperplasia (FNH), it has been reclassified as "telangiectatic hepatic adenoma" after recent studies indicated that it shares most molecular genetic and clinical features with conventional hepatic adenoma. Its monclonality has been proven by non-random inactivation of the X chromosome, methylation analysis of the HUMARA locus, and loss of heterozygosity in a genome-wide allotyping [10, 11].

Hepatic adenomatosis, which characteristically presents with more than 10 liver lesions, may run in a familial pattern, and has frequent germline mutations [12], and biallelic inactivation of hepatocyte nuclear factor 1α (*HNF1α*) [13].

Hepatocellular carcinoma

Hepatocellular carcinoma is the sixth most common malignancy and the third most common cause of cancer deaths world wide. The major risk factors include chronic viral infection (HBV and HCV), alcoholic/nonalcoholic liver disease, environmental carcinogens (i.e. aflatoxin B1 (AFB), and inherited genetic disorders (Wilson's disease, hemochromatosis, α-1-antitrypsin deficiency, and tyrosinemia) [14-17]. With a few exceptions, HCC always develops in the setting of chronic hepatitis or cirrhosis, in which there is continuous inflammation and regeneration of hepatocytes. The non-random accumulated genetic alterations or chromosomal aberrations during the processes of inflammation, regeneration, and cirrhosis lead to the development of HCC [18, 19]. This multi-step process starts from hyperplastic change, dysplasia, and early HCC, eventually resulting in full-blown HCC. Qualitative and quantitative genetic alterations precede each step of carcinogenesis.

Molecular basis of pathogenesis

HCC is a heterogeneous group of carcinomas, with largely diverse molecular alterations associated with different etiologies. HBV-associated HCC may occur in the liver without cirrhosis and is distinct from HCC related to HCV and other etiologies. Although it is still under debate, HBV may have both direct and indirect oncogenic effects on hepatocytes [20, 21]. One direct effect is that viral DNA may be integrated into the hepatocyte genome, causing disruption of chromosomal stability or tumor suppressor genes and activation of proto-oncogene [22]. Another direct oncogenic effect may be attributable to the 154-amino acid (16.5-kDa) viral protein HBx, which may transactivate or upregulate a variety of viral and cellular genes [23, 24]. The affected genes or molecules include basal transcription machinery in the nuclei (*TFIIB, TBP*, and *RPB5*) [25, 26], the Src pathway, and the Ras/Raf signaling pathway, which may, in turn, activate several oncogenes such as *c-myc, c-jun*, and *c-fos in* cytoplasm [27-30]. HBx protein amplifies TGF-β signaling through direct interaction with Smad4, and both direct

and indirect interactions with DNA repair protein UVDDB, tumor suppressor proteins (p53 and *APC* gene product), cell cycle regulators, growth factors and receptor genes, cytokines, genes involved in apoptosis [31], proteasome subunits [32-34], and NF-κB, a modulator of immune response [29]. Indirectly, HBV infection causes liver cell injury mediated by cellular immune responses, resulting in carcinogenesis by promoting cell death, proliferation, and genetic mutations. When the HBV envelope coding region is transferred with activated T-lymphocytes to stimulate immune response in transgenic mice, they not only develop chronic hepatitis, but eventually also develop HCC [35].

The carcinogenesis of HCV infection-associated HCC differs from that related to HBV infection, and is mainly due to the indirect effects of viral infection. HCV viral DNA is never integrated into the genome of hepatocytes[36], but HCV infection may cause the accumulation of genetic abnormalities during the degeneration-regeneration process. Viral proteins, viral core protein in particular, may interfere with intracellular signaling pathways (activating TNF-α receptor, Raf-1 kinase, and NF-κβ pathways, resulting in inhibition of TNF-α and Fas-mediated apoptosis) and interact with the host immune system [37, 38].

Exposure to food contaminated by Aflatoxin B1 (AFB1), a fungal metabolite produced by Aspergillus flavus and related fungi, is associated with an increased incidence of HCC [39, 40]. AFB1 and its metabolite may result in a high frequency of mutations affecting 249^{ser} in the *p53* tumor suppressor gene (codon 249 G:C to T:A transversion) [41-44]. Moreover, there is a dose-dependent relationship between *p53* 249^{ser} mutation load in cells and the intake of AFB1 in non-tumorous liver tissue [44]. In areas of the world with a high prevalence of AFB1 and HBV infection, synergy exists between HBV infection and high aflatoxin exposure in hepatocarcinogenesis [45].

The molecular mechanism for carcinogenesis associated with Wilson's disease, primary hemochromatosis, and other genetic diseases affecting the liver is also related to poorly controlled immune responses to copper or other metabolite accumulation. This immune response results in inflammation and generates oxidative free radicals that damage human DNA and cause genomic alterations in hepatocyte genes association with tumor suppression, cell cycle regulation, DNA repair, and apoptosis [46-48]. Mutation of *p53* was frequently observed at codon 220 (A-G) in British patients with hemochromatosis-associated HCC [49]. Another highly frequent *p53* mutation is at codon 249 (G:C to T:A transversion) in non-tumorous hepatocytes, and it is found in patients with hemochromatosis or Wilson's disease. Mutation at codon 250 (C:G to T:A transition) is also commonly seen in Wilson's disease-associated HCC [46]. The genetic abnormalities causing Wilson's disease or hemochromatosis do not increase the risk for carcinogenesis [50, 51].

Molecular genetics of precursors to HCC

Histopathologic and molecular biology studies have shown that the development of human HCCs is a multi-step process, from macroregenerative nodules (MRN)/low-grade dysplasia (LGD), to high-grade dysplasia (HGD), to early HCC [52-54]. After an initial exposure or insult by carcinogens, it may take years or decades for humans to accumulate the necessary genetic and epigenetic damages necessary for preneoplastic diseases to develop into HCC. These genetic damages or changes include the up-regulation of growth factors, inactivation of tumor suppressor genes, aberrant methylation, and microsatellite instability. Up-regulation of TGF-α and IGF-2 are sequelae of degeneration due to chronic inflammation, viral transactivation, and hepatocellular repair and regeneration [55]. Aberrant hypo- or hypermethylation observed in chronic hepatitis and cirrhosis as well as HCC is due to increases in DNA methyltransferases (DNMT) associated with chronic hepatitis and cirrhosis [56-59].

Loss of heterozygosity (LOH) and microsatellite instability occur in preneoplastic lesions and HCC [60, 61]. The gain at chromosomal locus 1q is the most common finding in dysplastic nodules and small HCCs [62]. Other informative markers include allelic chromosomal arms 1q and 14q, TATA box-binding protein (TBP) and BRCA1. LOH is detected in chromosome 8p21.3-p22 in approximately 40% of dysplastic nodules and HCCs. LOH on chromosome 11p13 is found in 15.8% of dysplastic nodules and 31.6% of HCCs. In dysplastic nodules, there is more LOH of D11S995 (33.3%) but less LOH of D11S907 (7.1%), whereas in HCCs, LOH of

D11S907 (44.4%) is more frequently found than that of D11S995 (8.3%) [63]. In general, the multiplicity of allelic deletions in affected cell populations is low in chronic hepatitis, rises in dysplastic lesions, and is highest in HCCs [62, 64]. Gene profiling analysis in comparison to normal or surrounding cirrhotic tissue demonstrates that among the cDNA of 1152 genes tested, MRNs and dysplastic nodules have over 50 genes that are consistently deregulated. These deregulated genes (29 up-regulated and 24 down-regulated) include oncogenes, tumor suppressor genes, DNA repair genes, genes encoding cell growth factor and cytokines, genes encoding adhesion proteins, signal transduction genes, transcription factors, transcription factor/DNA binding protein genes, and housekeeping genes [65].

The unique molecular alterations seen in HCC precursor lesions may be useful for early diagnosis. However, several studies showed that the genetic or genomic alterations in preneoplastic or dysplastic nodules may not necessarily be found in HCC cells. These genetic or genomic differences suggest that not every early genomic aberration in precursor lesions is necessary or sufficient for the induction of malignant transformation of hepatocytes [66, 67]. Thus, most molecular alterations seen in preneoplastic lesions may not be suitable for diagnostic purposes. Although there are several candidate molecular markers (i.e. HSP70, CAP2, glypican 3 and glutamine synthetase) that have proven useful for the histologic diagnosis of early HCC, these results have yet to be confirmed in routine pathologic diagnosis [68].

Molecular genetics of HCC

Chromosomal abnormalities: Genomic abnormalities in HCC are largely heterogeneous due to the different molecular mechanisms of carcinogenesis related to different etiologies and the multifactoral process of oncogenesis. Gain of chromosome 10q is unique to HCV-related HCC, while loss of 4q and 16q and gain of 11q are seen preferentially in HBV positive cases [62, 69]. Conventional cytogenetic studies and CGH have shown that most HCCs are aneuploid and harbor multiple chromosomal abnormalities, including non-random, recurrent DNA copy number losses on multiple chromosomal arms (1p, 4p, 5q, 6q, 8p, 9p, 13q, 16p, 16q, 17p) and gains on others (1q, 6p, 8q and 17q) [4, 70-74]. Chromosome 1q is the most common aberration across different geographic locations [72-74]. The frequently deleted chromosome regions by LOH in HCCs contain many tumor suppressor genes and some oncogenes, (*p53, Rb*, *p16*, *PTEN*, *DLC*1, and *IGF2R)* [78-81]. LOH at chromosome 1p is usually seen in early, small or well-differentiated HCC [82], whereas LOH at chromosomes16p and 17p is more frequently associated with HCCs in advanced stages, aggressive tumor, and poor prognosis [83, 84]. By CGH, chromosome 8p, 17p and 19p are associated with HCC metastases [85].

Deregulation of signaling pathways: Deregulation of the major signal transduction pathways is found in all HCCs but differs with associated etiology. Abnormal activities of Wnt/β-catenin, hedgehog signaling, TGFβ, MAP/ras, IGF, apoptosis, microsatellite stability, phosphatase and tensin homolog gene (*PTEN*), *p53*, and Rb1 pathways are commonly found in HCCs, irrespective of etiology, and probably reflect common pathogenic mechanisms such as chronic liver injury and cirrhosis [9, 78, 86]. However, HCCs of different etiologies may predominantly affect certain pathways. HCV-associated HCC shows significant abnormalities in both Wnt/β-catenin and MAP kinase pathways [87, 88]. Dysfunction of Wnt/β-catenin, p53, pRb, MAP kinase [89], and cytokine signaling is more commonly seen in HBV-related HCC [87, 88, 90, 91]. Tumors associated with alcoholism have more frequent alterations in the Rb1 and p53 pathways than those caused by HCV infection [92]. The "aflatoxin-associated" *p53* mutation in codon 249 is identified only in samples from areas with high aflatoxin content (Asia and Africa) [93, 94].

Abnormal Wnt signaling in HCC is exemplified by β-catenin overexpression or activation. β-catenin plays an important role in both intercellular adhesion and differentiation. Mutations in the *β-catenin* gene are detected in 26-41% of HCCs [95-97]. It is clinically related to less aggressive tumors than those without this mutation but harboring multiple chromosomal aberrations [98].

Deregulation of the p53 pathway is the most common cause of human carcinomas, including HCC. Loss of *p53* function is observed in 25-60% of tumors [99] and occurs mostly due to allelic deletions at chromosome 17p13 and

missense mutations in the specific DNA-binding domain [66, 100, 101]. *p53* mutation is probably a late event in oncogenesis and is associated with both progression of HCC from an early to a more advanced stage [99, 102, 103] and HCC recurrence [104, 105]. A downstream target of zinc finger transcription factor ZBP-89 can be co-localized with p53 in the nucleus and appears to help nuclear accumulation of the p53 protein in a subset of recurrent HCC. Co-localization of p53 protein with ZBP-89 may define a subgroup of recurrent HCCs that are more sensitive to radiation or chemotherapy [106].

Retinoblastoma pathway inactivation is mainly through RB1 and CDKN2A promoter methylation and rare genetic mutations. LOH at the *Rb* locus has been found in 25-48% of cases and strong down-regulation is seen in up to 50% of cases [79]. *Rb* gene is an important cycle controller and can be inactivated by mutations in the gene itself, loss of TGF-β responsiveness, and inactivation of *p16*, *p15,* or *CDK*4 [82, 107]. Loss of p16 protein due to inactivation of *p16* by promoter hypermethylation, homozygous deletions, and point mutations may be noted in both early and late stage of HCC [108].

Microsatellite instability occurs in HCC as well as chronic hepatitis and cirrhosis [60, 61]. The incidence of microsatellite instability is higher in European HCC and in liver cirrhosis associated with HBV infection. In other parts of the world, microsatellite instability is an infrequent event [109] and only 11% of HCCs have abnormal DNA repair function. The degree of this abnormality correlates significantly with poor differentiation and portal vein involvement of HCC [110].

The Met pathway is deregulated in a subset of human HCCs. MET is an oncogene that encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF) located on chromosome 7q21-q31. A subset of human HCCs with deregulated Met expression shows aggressive phenotype and poor prognosis [111].

Gene expression profiling of HCC: Recently developed technology, such as DNA microarrays and other molecular profiling techniques, has provided new insights into the molecular genetics of HCC [112-122]. Data from these techniques have demonstrated that, in most cases, transcripts that either directly or indirectly promote cell proliferation/growth are upregulated and those that inhibit cell proliferation/growth are downregulated. Many different cellular pathways are affected by these deregulated genes and gene products, including the extracellular matrix, the cytoskeleton (MMP14), oncogenes (*Rho*, *raps* homolog gene), tumor suppressor genes, MHC class IC or HLA-C, apoptosis-related genes (*Dynein*), signal transduction/ translational regulator genes (Wnt/ β-catenin pathway members), and genes related to biotransformation/metabolism (*GST*, monoamine oxidase, cytochromes, etc.). Moreover, gene expression profiling data confirm that HCV-related HCCs have different molecular genetics from those associated with HBV-related HCCs [123, 124], supporting the theory that these disease processes are driven by different pathophysiological mechanisms of hepatocarcinogenesis. These genes or gene products may be used as potential tumor markers that can be readily detected by serological or molecular tests. In addition, some gene profiling or signature genes have been found to be associated with greater potential for metastasis and recurrence [117, 125, 126].

Aberrant expression of MicroRNAs: MicroRNAs are small, noncoding RNAs with a stem–loop structure that are initially produced by RNA polymerase II. They usually bind to 3′ untranslated regions of mRNA transcripts to regulate gene expression. Aberrant expression of several miRNAs has been implicated in HCC carcinogenesis, and miRNA expression signatures correlated with pathological and clinical behavior of HCC. Up-regulation of mir-221 and mir-21 could reduce tumor apoptosis and lead to angiogenesis and invasion [127]. Receptor tyrosine kinase RAS and PI3K pathways are affected not only by down-regulated miR-1, miR-199a, and Let-7, but also by upregulated miR-2. Results may lead to cell growth, survival, motility, invasion, and metastasis [128-130]. A 20-miRNA signature has been found to be associated with HCC venous invasion and could also correlate with disease free and overall survival time [131].

Molecular classification and prognostication

Current classification and staging of HCC is mainly based upon histomorphology and/ or associated etiology and clinical presentation. In general, these classification or staging systems

can provide useful information for the management of patients, prognostication, and to some extent, therapy, but their clinical relevance and accuracy are debatable. It has been advocated that molecular approaches, such as gene expression microarray and SNP array, should be used to develop a new classification system for HCCs that better predicts clinical outcome and facilitates targeted molecular therapy [132-135].

Expression signatures found via global gene expression profiling can stratify HCCs into several clinically relevant groups. For example, using DNA microarrays containing 21,329 unique genes, 91 human HCCs were analyzed; these data subclassified HCC into two distinctive groups. Class A, a low survival subclass (overall survival time 30.3+- 8.02 months), shows a strong expression signature of cell proliferation and anti-apoptosis genes (such as *PNCA* and cell cycle regulators: *CDK4*, *CCNB1*, *CCNA2*, and *CKS2*) as well as genes involving ubiquitination and sumoylation. In comparison, Class B, a high survival subclass (overall survival time 30.3+- 8.02 months 83.7 +-10.3 months), does not have the above expression signature[136]. Furthermore, gene expression profiles of nontumoral liver tissue from paraffin-embedded specimens can be used to subclassify HCC patients into different survival groups [133].

A genome-wide transcriptomic analysis of 60 HCC tumors found 16 gene signatures that classify HCC tumors into the six robust subgroups (G1–G6). Each subgroup has unique clinical and genetic characteristics based upon chromosome stability status: G1–G3 are chromosome unstable and G4–G5 are chromosome stable. Since each group of tumors has specific pathway activations (i.e., protein kinase B (AKT or PKB)) in G1-G2 and Wnt pathways in G5-6), this molecular classification can not only provide prognostic information, but also facilitate the development of targeted therapies for HCC [132].

Whole-genomic array CGH analysis of 87 HCCs revealed two groups of tumors (clusters A and B) with significant differences in chromosomal alteration profiles and clinical outcomes. Cluster A's progression is more malignant than cluster B, shows exclusive chromosomal amplifications on 1q, 6p, and 8q, and has chromosomal losses on 8p. Cluster B has a low frequency of chromosomal alterations and tends to harbor limited numbers of chromosomal alterations. Since HCC is composed of several genetically homogeneous subclasses with characteristic genetics, these data have illuminated the opportunity for using targeted molecular therapy according to specific genetic background [134].

Molecular therapeutic targets of HCC

Treatment options for early or small HCC include liver transplantation, resection, or local radiation therapy, which significantly improve patient survival. However, because patients with HCC are usually diagnosed at advanced stages of disease, the above treatment modalities and chemotherapy are rarely effective. Furthermore, there is significant clinical and genetic heterogeneity among HCCs of different etiologies, thus one or a few standard treatments may not work for all HCCs. Recently introduced molecular targeted therapies are specific for groups of HCCs with similar genetics. The targeted therapy aims to inactivate activated oncogenes, recover tumor suppressor genes, or repair other genes and molecules related to HCC development, thereby correcting abnormal genes or functions as well as biological behavior. Recently, many candidate genome-based drug targets have been discovered via microarray technology, whole-genome epigenetic aberration analysis using promoter arrays, ChIP-chip analysis, and high-throughput sequencing systems. Examples of target genes or molecules include VEGFR, EGFR, *DDEFL, VANGL1, WDRPUH, Ephrin-A1,* gypican-3 (GPC3), number gain 7q, PFTAIRE protein kinase 1 (PFTK1), paternally expressed 10 (PEG10), and miR-122a [137-147]. Moreover, some of these targeted therapies, such as monoclonal antibodies, small molecules and antisense drugs, have reached phase II and III clinical trials for therapeutic use, and many have been shown to be effective. Sorafenib, an oral multikinase inhibitor of vascular endothelial growth factor receptor (VEGFR) and Ras kinase, has been approved by the FDA as a molecularly targeted anticancer agent [138, 148]. Some other agents targeting similar genes or molecules are being tested in preclinical and clinical trials for HCC. Results are summarized below and partially listed in **Table1**.

1. Anti-Epidermal growth factor receptor (anti-EGFR) therapy: Epidermal growth factor receptor (EGFR) is frequently expressed in both hu-

Table 1. Current molecular targeted therapies in HCC

Drug	Type of Drugs	Molecular Targets	Affected Signaling Pathways	FDA Approval
Sorafenib	Tyrosine kinase inhibitor	VEGFR, PDGFR, RAF	VEGFR, PDGFR, RAS/MAPK	yes
Sunitinib	Tyrosine kinase inhibitor	VEGFR, PDGFR, c-kit	VEGFR, PDGFR, c-kit	No, phase II or 3 trials
Bevacizumab	Monoclonal antibodies to ligand	VEGFR	VEGFR	No, phase II or 3 trials
Cetuximab	Monoclonal antibodies to ligand	EGFR	EGFR	No, phase II or 3 trials
Erlotinib	Tyrosine kinase inhibitor	EGFR	EGFR	No, phase II or 3 trials
Gefitinib	Tyrosine kinase inhibitor	EGFR	EGFR	No, phase II or 3 trials
Lapatinib	Tyrosine kinase inhibitor	Her-2/neu	Her-2/neu	No, phase II or 3 trials
Rapamycin	ST kinase inhibitor	mTOR	PIK3/Akt/mTOR	No, phase II or 3 trials
Everolimus	ST kinase inhibitor	mTOR	PIK3/Akt/mTOR	No, phase II or 3 trials
XL-765	ST kinase inhibitor	PI3K	PIK3/Akt/mTOR	No, phase II or 3 trials
Trastuzumab	monoclonal antibodies to receptor	Her-2/neu	Her-2/neu	No, phase II or 3 trials

man HCC cell cultures and tumor tissues. Monoclonal antibodies against EGFR, such as Cetuximab, and small molecule tyrosine kinase inhibitors, such as Gefitinib and Erlotinib, have shown therapeutic effects in both cell culture and in patients in a phase II study [137].

2. Anti-vascular endothelial growth factor (antiangiogenesis): Vascular endothelial growth factor (VEGF) is upregulated via 6p21 gain [135] in HCC and targeting VEGF in HCC has potential anti-angiogenic effects. It is one of the putative targets of Sorafenib, an oral inhibitor of the VEGF receptor and other kinases [135]. Administration of this drug in patients with advanced HCC was shown to increase median overall survival in a phase III, randomized, placebo-controlled trial (SHARP trial) [149]. Bevacizumab, a recombinant, humanized monoclonal antibody, inhibits VEGF and also decreases the permeability of tumor vessels and relieves elevated tumor interstitial pressure, thus potentially enhancing the effectiveness of chemotherapy [150, 151].

3. Multikinase targets: Many reagents target multiple sites of pathways or multiple genes or products. Sorafenib inhibits both VEGF and either K-ras or its downstream effectors in the RAF/MEK/ERK pathway, thereby inducing tumor cell apoptosis [152]. Similar to Sorafenib, Sunitinib is an oral and multi-targeted receptor tyrosine kinase that exerts an antiangiogenic effect by targeting the tyrosine kinases VEGFR and platelet derived growth factor receptor (PDGFR). It has shown anti-HCC activity in both xenograft models and in a phase II clinical trial in patients with unresectable or metastatic HCC [153 - 155].

In summary, targeted therapy has proven to be an effective treatment for certain groups of HCC patients who might not respond to conventional therapeutic modalities. With a better understanding of the molecular mechanisms of hepatocarcinogenesis, more therapeutic options will be offered to cure or alleviate the symptoms of HCC.

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