

Hepatocellular Carcinoma: Molecular and Genomic Guideline for the Clinician

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KEYWORDS

- Hepatocellular carcinoma • Multikinase inhibitors
- Targeted therapy • Molecular classification

Conventional approaches to prognostic classification and treatment recommendations for the worldwide burden that is hepatocellular carcinoma (HCC)¹ have largely relied on clinicopathological characteristics, such as tumor size, Child-Turcotte-Pugh score, serum levels of α -fetoprotein (AFP), number of nodules, functional performance estimates (such as the Eastern Cooperative Oncology [ECOG] performance status), vascular invasion, distant metastases, and tumor grade. With improved DNA-microarray methods, defining the genomic and molecular changes that occur during the development of HCC should improve the classification and prognostication of liver tumors. Additional testing with RNA and proteomics will further add to the profiling of HCC. The development of sorafenib and other new targeted therapies in development has been made possible by the discovery and understanding of the molecular and genetic pathogenesis of hepatocellular carcinoma, and the signaling pathways that are important in the progression of the disease from dysplastic lesions to cancer and further progression of disease clinically. The understanding of these pathways will in the future enable the clinician to focus the treatment patients with HCC and customize single or combination therapy and allow refinement of treatment sequencing. Furthermore, investigators will be able to understand the basis of newer therapies that need to enter investigational trials. The discussion that follows complements the information in articles elsewhere in this issue, all in relation to HCC, by

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Roskams on Anatomic Pathology, Siddique and Kowdley on Insulin Resistance and Other Metabolic Risk Factors, the Diagnostic Role of Tumor Markers and Liver Biopsy by Gonzalez and Keefe, the Role of Oncogenic Viruses by Zemel, Isaschar, and Tur-Kaspa, and Systemic Therapy by Wrzenski, Taddei, and Strazzabosco.

PATHOGENESIS OF HCC

Development of hepatocellular carcinoma involves a complex, multistep pathway where normal hepatocytes proliferate in the setting of chronic inflammation and altered intracellular signaling, and subsequently develop into regenerative nodules, with evolution to dysplastic nodules and cancer.² Most often, this occurs in a chronically diseased liver caused by hepatitis B virus (HBV) or hepatitis C virus (HCV). The most important molecular event that occurs in cancer development is aberrant gene expression. Genetic and epigenetic changes may occur, such as direct integration into the cellular DNA and “injury” with genetic mutations and translocations; or epigenetic modulations of genes such as DNA methylation, which results in altered gene expression without any changes in the base sequence of DNA, can occur.³ Modifications of DNA sequences that do not directly encode mRNA and proteins can modify the generation of micro-RNA (miRNA); this can then result in dysregulation of primary gene signaling.⁴ Cancer cells do not invent new pathways of signaling, but instead use preexisting pathways that are dormant or less active in normal cells. The changes in genetics, epigenetics, or miRNA allow these pathways to become overactive in cancer cells, increasing cell proliferation and signaling and decreasing cell apoptosis, with escape from the normal growth inhibition and regulation of growth from the extracellular matrix.

The pathogenesis of hepatocellular carcinoma is incredibly complex, since the development of a malignant signature can occur with several different mechanisms. This process results in different subtypes of HCC that are similar on pathologic examination, but clinically behave in different manners. For example, HCC related to HBV infection can have the HBV DNA (full or partial sequences) incorporated into many different areas in the host DNA, whereas the HCV virus does not incorporate into the host DNA and the development of HCC is related to decades of liver cell damage and regeneration.³ HCV, through oxidative injury, can also result in cellular DNA modification.

Elevated expression of transforming growth factor (TGF)- α and insulin-like growth factor (IGF)-2 are both responsible for accelerated hepatocyte proliferation.⁵⁻⁷ This increased expression reflects the expression of several genes that are upregulated by epigenetic mechanisms during the preneoplastic stage, resulting from the combined actions of cytokines produced by chronic inflammation, viral transactivation, and the regenerative response of the liver to cell loss. As hepatocytes repeatedly proliferate in response to these signals, monoclonal hepatocyte populations develop with progressive telomere shortening that predisposes to the development of dysplasia.

GENETIC ALTERATIONS

Nearly all liver cancer cells contain many genes and genetic loci that have structural alterations, but specific genetic or allelic alterations studies rarely affect more than half of liver cancers studied. The amount of variation among these genetic alterations has prompted the development of genetic and molecular subclassifications of HCC.

Many different genetic alterations have been discovered in HCC, including gains in chromosomes 1q, 6p, 8q, 17q, and 20q, and losses in 4q, 8p, 13q, 16q, and 17p.⁸

Mutations in the tumor suppressor gene p53 occur frequently in human cancer development, but are only found in 29% of HCC. In addition, HBV-induced HCC is also closely linked to p53 alterations.⁹ Expression of HBV viral protein HBX inactivates p53-dependent activities, a tumor suppressor gene, which includes p53-mediated apoptosis.^{10,11} Activation of oncogenes such as k-Ras seems to occur at a lower rate than in other human cancers, only occurring in 3% to 10% of liver cancers.¹² Other oncogenes are thought to be more important, such as c-met, which was shown to be expressed in 40% of HCC.¹³

Knowing what genetic alterations have occurred can help to assess the prognosis of disease. In tumors with a p53 mutation, loss of heterozygosity (LOH) of 17p was found in 95% of liver cancers.⁹ These tumors are more often poorly differentiated and at a more advanced stage of disease at diagnosis.⁹ Many tumor suppressor genes on several different chromosomes have been found to have LOH, including 17p (p53), 13q (RB1, retinoblastoma 1), 16p (AXIN1, axis inhibition protein 1), 9p (CDKN2A, cyclin-dependent kinase inhibitor 2A), and 6q (IGF2R, insulin-like growth factor 2 receptor).³ These changes can result in further inactivation of tumor suppressor genes, and signal the development of malignant cell development.

Another tumor suppressor gene, retinoblastoma gene Rb, which is located on chromosome 13q14, also appears to be involved in HCC development, with 43% of tumors showing alteration in this gene.¹⁴ Inactivation of Rb contributes to tumor progression and metastasis in many patients rather than tumor initiation, and therefore tends to occur at more advanced stages of disease.¹⁵

LOH on chromosome 16, corresponding to tumor suppressor gene AXIN1, occurs in more than 50% of cases of HCC, and occurs more frequently in large, poorly differentiated, and metastatic liver cancers, but not early liver cancers.³ This LOH also plays a role in tumor aggressiveness rather than tumor initiation.

DNA methylation is a key process in the epigenetic control of gene expression. Hypermethylation can lead to the inactivation of tumor suppressor genes and invasion-suppressor genes, and predisposes for allelic loss.^{16,17} Hypomethylation of DNA increases the expression of oncogenes.¹⁸ The level of DNA methylation is controlled by DNA methyltransferase, which transfers a methyl group from S-adenosylmethionine (SAMe) to the 5-position of cytidine. Increased expression of DNA methyltransferase is another step involved in human carcinogenesis.¹⁹ Of interest, DNA methyltransferase mRNA levels are significantly higher in liver tissue showing chronic hepatitis or cirrhosis than in normal liver tissue, and are even higher in HCC cells.^{20,21} This finding suggests that increased DNA methyltransferase expression is also a step that is responsible for hepatocarcinogenesis, especially in the early stages of disease.

Changes in DNA methylation are reversible, and correcting this aberrant process seems to be more feasible than correcting genetic alterations such as gene mutations. Reducing DNA methyltransferase activity has been shown, in experimental models, to inhibit tumor development, and thus may be a potential target for the prevention and subsequent treatment of HCC.^{22,23}

Microsatellite instability also occurs in hepatocytes as a preneoplastic lesion. In the early preneoplastic stage, structural alterations in DNA occur slowly, but as dysplasia and neoplasia develop the rate of development of structural alterations and amount of alterations increase sharply.²⁴ Individual liver cancers often contain multiple allelic deletions and chromosomal losses and gains concurrently, which emphasize the complexity of HCC development. Through the progression of HCC from small, well-differentiated tumors to large, poorly differentiated, and metastatic tumors, further chromosomal alterations occur, resulting in a tumor with areas of diverse mixtures of genomic aberrations.²⁵

GENETIC CLASSIFICATION OF HCC

Many genetic alterations accumulate during HCC development and may involve more than 20 different genes, with at least 3 and probably more different signaling pathways affected, as follows:

1. The Wnt pathway, a term derived from the genetic mutation in *Drosophila melanogaster* fruit fly, which produces a wingless phenotype. The *wingless* gene is a homolog to the *int-1* gene of vertebrates, which was originally identified near insertion sites for mammary tumor virus in mice where virus integration causes amplification of *Wnt* genes. Wnt is a catenation of *wingless* and *int*.
2. The extracellular signal-related kinase, or ERK, pathway
3. The mammalian target of rapamycin, or mTOR, pathway.

The goal of discovering particular genetic and molecular alterations is to apply the knowledge to clinical management and treatment of HCC. As such, several different groups have attempted to first classify HCC into different groups based on the etiology, clinical characteristics, and aggressiveness, and then to identify the associated genetic alterations. For instance, Laurent-Puig and colleagues²⁶ analyzed a large number of genetic alterations in the same series of tumors, which enabled the definition of two mechanisms of HCC development.²⁶ These investigators found that LOH was the most frequent genetic alteration, and half the tumors exhibited LOH on more than 5 chromosome arms (Pathway I). In the other half of HCCs, tumors were chromosome stable, and the β -catenin mutation was the one most frequently observed (Pathway II). The tumors in Pathway I were predominantly from HBV-infected patients, were poorly differentiated, and had a poor prognosis. Pathway II tumors were generally non-HBV-infected, and were large and well differentiated.

Another genetic classification system was developed by Kato and colleagues.²⁷ These researchers analyzed chromosomal alteration profiles, named cluster A and cluster B, that were associated with viral infection status, serum AFP levels, and presence of intrahepatic metastasis, as well as patient survival. All liver cancers had chromosomal alterations to varying degrees, but more frequent and pronounced alterations were observed in cluster A, which had a worse prognosis and poorer survival than cluster B (Fig. 1). In addition, subclusters were noted that may respond

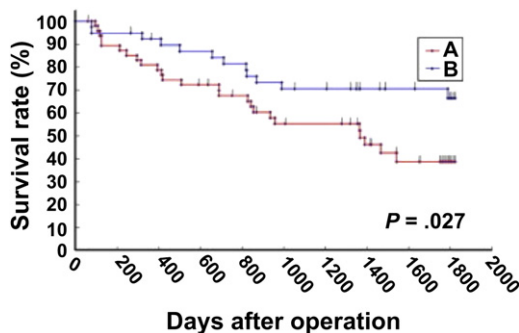


Fig. 1. Kaplan-Meier survival curve demonstrating survival difference after partial hepatectomy in patients with hepatocellular carcinoma, with stratification based on chromosomal alteration profiles. (From Kato H, Ojima H, Kokubu A, et al. Genetically distinct and clinically relevant classification of hepatocellular carcinoma: putative therapeutic targets. *Gastroenterology* 2007;133:1480; with permission.)

well to different treatments, such as a subcluster A1 that was characterized by amplification on 1q and/or 6p, and which may respond to molecular inhibitors available for vascular endothelial growth factor (VEGF). A subcluster A3 showed a higher rate of 17q gain/amplification, and results of *in vitro* experiments show that these cell lines respond well to rapamycin, an mTOR inhibitor that directly inhibits the activity of S6 kinases.²⁸ These data suggest that molecular stratification of individual liver cancers into genetically homogeneous subclasses could provide an opportunity for developing optimal tailor-made therapeutic agents.

A third genetic classification system has been developed by Boyault and colleagues.²⁹ This classification also found 6 main subgroups based on genetic alterations in the tumors. The primary clinical determinant of class membership in this scheme is HBV infection, with other main determinants being genetic and epigenetic alterations, including chromosomal instability, β -catenin and p53 mutations, and parental imprinting.

More than 6 different groups have attempted to develop HCC classification systems based on genetic alteration of the tumor cells.^{27–34} The fact that so many different groups have attempted to classify HCC highlights the importance and difficulty of understanding the genetic basis of hepatocellular carcinoma. All of the genetic classification systems have similar findings, notably that HBV infection, chromosomal instability, and changes in β -catenin and p53 seem to be the most important factors in tumor behavior. The classification systems can identify tumors that are at high risk for being poorly differentiated histologically, having vascular invasion, and having poor patient survival. With further investigation, these classification systems may be used to determine the targeted therapies that would be best suited to particular patients in each unique subclass of HCC.

NONTUMOR TISSUE AND PROGRESSION OF HCC

In addition to the genetic alterations in tumor tissue, when HCC is resected the genetic alterations in the tissue adjacent to the tumor also seems to affect survival. Hoshida and colleagues³⁵ analyzed 186 gene signatures, 113 of which were associated with a good prognosis and 73 with a poor prognosis. Early recurrence of tumor is associated with clinical and histopathological factors associated with the resected tumor, but late recurrence (more than 2 years after resection) is associated with the gene-expression signature of the nontumoral liver tissue. This finding indicates that late occurrences are likely to be new primary tumors rather than actual recurrences. The poor-prognosis signature contained gene sets that are associated with inflammation, such as interferon signaling, activation of nuclear factor kappa B (NF- κ B), and signaling by TNF- α .

The impact of inflammation on the risk of metastasis remains unclear. Budhu and colleagues³⁶ investigated the impact of inflammatory changes on the risk of metastasis in HCC. Using two gene clusters, patients with metastatic disease were found to have a significantly different microenvironment in nontumor tissue compared with patients without metastatic disease. Unlike the findings of Hoshida and colleagues, where inflammation conferred a poor prognosis, the gene set investigated by Budhu showed that patients without metastasis seemed to have an increase in inflammatory cytokines compared with patients with metastatic disease. In fact, patients with metastatic disease had a global decrease in proinflammatory cytokines.

The use of nontumoral gene expression profiling may be used to identify patients at the highest risk of recurrence in order to target intensive follow-up or use of adjuvant therapies. However, there remain many unanswered questions as to the true genetic

changes that will offer the best profiling available, and further research is needed before clinical tests that can be applied to patient care can be embraced.

MICRORNAS

miRNAs are small, noncoding RNA gene products that play key regulatory roles in mRNA translation and degradation.^{37,38} In addition, each miRNA has the capability to regulate the expression of hundreds of coding genes and modulate multiple cellular pathways including those for proliferation, apoptosis, and stress response.³⁹ miRNAs are frequently produced from “fragile” DNA sites, common break points, or regions of amplification or LOH. Research has clearly shown that miRNAs play a significant role in human hepatocarcinogenesis.⁴⁰ Mature miRNAs are relatively stable; this characteristic may make miRNAs excellent molecular markers that may be used as a tool for further cancer diagnosis and prognosis. Budhu and colleagues⁴ analyzed a 20-miRNA signature that could significantly predict metastatic (M) from nonmetastatic (NM) status with an accuracy of 76%. In this cohort of patients with early-stage HCC, the patients with M status had a 500-day survival rate of 38%, compared with 73% 500-day survival in patients with NM status. This outcome was related to the presence of metastatic disease and higher recurrence rate in patients with miRNA profiles consistent with M status. Ji and colleagues⁴¹ investigated miRNA patterns in 455 resected HCC specimens, and found that overexpression of miRNA-26 was associated with overall survival, while lower expression was associated with response to interferon in the adjuvant setting. Other important miRNAs are miR-122 and miR-221, both of which are associated with a poor prognosis as well.^{42,43} Many other miRNAs are being studied to elucidate their roles in liver cancer. The ability to understand the alterations in miRNA expression in liver cancer may be used in the future to accurately predict prognosis and potentially offer adjuvant therapy.

MOLECULAR PATHWAYS

HCC has traditionally been considered a chemotherapy-resistant tumor. However, in 2007, with the approval of sorafenib, for the first time a systemic targeted therapy was accepted as standard of care in the treatment of HCC.⁴⁴ In many cancers, specific drugs are indicated for specific subtypes of cancer. For instance, trastuzumab (Herceptin) is only effective in patients with breast cancer who express the erythroblastic leukemia virus oncogene homolog 2 (ERBB2), also known as the human epidermal growth factor (HER)-2 gene, as is imatinib (Gleevec) for patients with chronic myeloid leukemia resulting from the breakpoint cluster region (BCR)-Abelson leukemia virus (ABL) gene fusion.^{45,46} With improved knowledge of specific pathways that are upregulated in HCC, targeted therapies for the subtypes of HCC will also be able to be employed in the future.

The Wnt/ β -catenin signaling pathway, many other receptor tyrosine kinases activating downstream of Ras/Raf/MAPK or ERK kinase (MEK)/ERK signaling, and the Akt/mTOR signaling pathways have all been identified to be important in liver cancer, and would serve as markers of cancer activation and targets of therapy.^{7,47–49} Other molecular signals are important in stimulating tumor signaling pathways, including epidermal growth factor (EGF), VEGF, IGF, fibroblast growth factor (FGF), and others (Fig. 2). These molecular signals are current targets of therapy that are in development for cancer treatment not only in liver cancer but in many other cancers as well (Table 1).

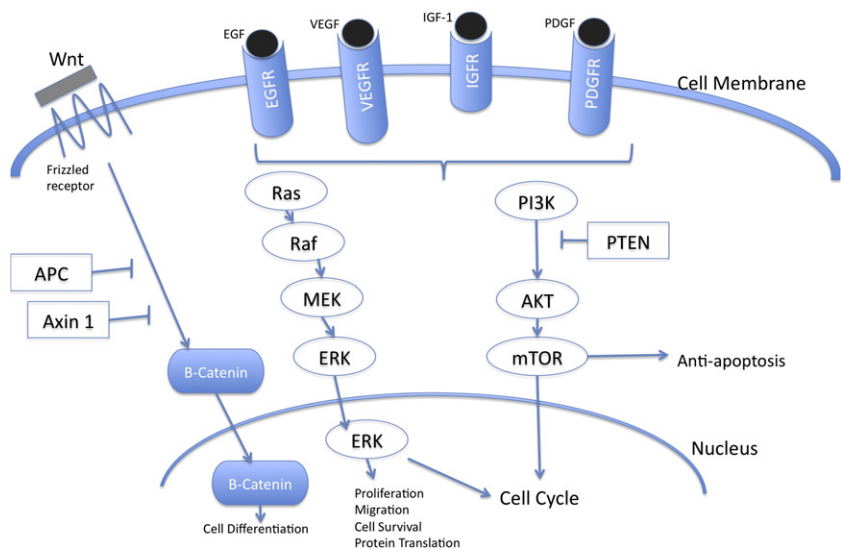


Fig. 2. Summary of signaling pathways and stimulating growth factors important in hepatocellular carcinoma. Cell membrane receptors: EGF, epidermal growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor. See text for explanation of the remaining acronyms.

Table 1 Current targeted therapies in clinical use or under investigation		
Drug	Molecular Target	Cancer
Sorafenib	VEGFR, PDGFR, raf	Liver, renal
Brivnib	VEGFR2, FGFR	Liver, solid organ, colon
Erlotinib	EGFR	Liver, lung, pancreatic
Linifanib	VEGFR, PDGFR	Lung, liver
Sirolimus	mTOR	Liver, Kaposi's, renal
Sunitinib	VEGFR, PDGFR, cKIT	Renal, GIST
PI-88	FGF, VEGF	Lung, liver
Trastuzumab	HER2	Breast
Rituximab	CD20	Lymphoma
Imatinib	BCR-ABL, cKIT	CML, GIST
Cetuximab	EGFR	Colon, head/neck
Bevacizumab	VEGF	Colon, lung, breast
Dasatinib	BCR-ABL, src	CML
Lapatinib	HER2, EGFR	Breast

Abbreviations: BCR-ABL, breakpoint cluster region-Abelson leukemia virus; CML, chronic myelocytic leukemia; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GIST, gastrointestinal stromal tumor; HER2, human epidermal growth factor 2; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Ras Sarcoma (Ras) Pathway

The Ras pathway is a dominant signaling pathway promoting cell proliferation and survival. The binding of growth factors (EGF, IGF-1, and platelet-derived growth factor [PDGF]) to their receptors activates Ras, which in turn activates Raf, MEK, and ERK. The phosphorylated ERK in the nucleus activates transcription factors such as c-Jun, which regulate the expression of genes involved in proliferation and survival.⁵⁰ Aberrant activation of the Ras/Raf pathway can occur at multiple different levels, and has been shown to be upregulated in multiple solid tumors. Newell and colleagues⁵¹ performed extensive genomic analysis in a cohort of 351 tumor samples, and found several different methods of Ras signaling in HCC. Overexpression with H-Ras, DNA copy number gains with B-Raf, and aberrant methylation of key genes in this pathway are all different ways in which the Ras pathway may be upregulated in HCC. In addition, these investigators identified MAP2K4, which is a negative regulator of Ras signaling and may be a new candidate tumor suppressor gene in HCC.

Sorafenib is the first multikinase inhibitor approved for the treatment of nonresectable HCC. Sorafenib blocks the Ras/Raf pathway at multiple levels, and has been shown to improve survival in patients.⁵² It is the first drug to follow the proof of concept that blocking upregulated pathways in hepatocellular carcinoma may improve tumor response to systemic therapy. The ability of sorafenib to increase survival of patients with liver cancer seems to be related to its ability to block the Ras/Raf pathway. In patients with liver cancer, ERK tissue concentrations are upregulated, and the level of upregulation seems to be related to the response of sorafenib.⁵³

Wnt/ β -Catenin Pathway

β -Catenin has a dual function in adhesion and Wnt signaling. It can acquire oncogenic activity as the result of mutations within its N-terminus.⁵⁴ These mutations result in loss of negative regulation of β -catenin, resulting in high cytoplasmic and nuclear levels, which then activate the transcription of Wnt target. In HCC, β -catenin mutations are found in 13% to 43% of tumors, and are mutated in some adenomas with a higher risk of malignant potential.^{20,55–58} In addition, HCV has been shown to act as a Wnt ligand to upregulate cell signaling pathways, and causes mutations in β -catenin.^{59,60}

The Wnt pathway may also be activated by several other methods. Inactivation of the tumor suppressor gene adenomatous polyposis coli or upregulation of the frizzled-7 receptor also causes activation of the pathway.^{61,62} The AXIN1 gene noted earlier is also important in the Wnt pathway. It codes for a protein of a complex that inhibits the Wnt pathway, and mutation with subsequent inactivation of this gene results in activation of Wnt target genes.⁶³

Activation of the Wnt signaling pathway has many carcinogenic properties, such as increased gene products responsible for proliferation (myc), activation of oncogenes (such as c-myc and cyclin D), antiapoptosis (survivin), invasion (matrix metalloproteinases [MMPs]), and angiogenesis (VEGF).⁶³ Thus, activation of Wnt not only regulates tissue development but also influences tumor growth and progression.

At present there are no therapies available that target this pathway; however, there are some molecules in preclinical trials that interfere with various locations in this pathway.^{64,65}

Akt/mTOR Signaling

Another important signaling pathway in HCC is the Akt/mTOR signaling pathway. This pathway is frequently amplified and overexpressed in various cancers, and is

activated in approximately half of patients with liver cancer.⁶⁶ Akt is a serine/threonine kinase that acts as a cytoplasmic regulator of numerous signals related to cell cycling (cyclin D1), cell survival (Mdm2/p53), cardiovascular homeostasis (endothelial nitric oxide synthase), and cell growth (mTOR).⁶³ The tumor suppressor phosphatase and tensin homolog (PTEN) is a negative regulator of the pathway, and its loss activates Akt.

mTOR complex 1 (mTORC1) is a downstream target of Akt that acts as a central regulator of cell growth and proliferation by activating S6 kinase, which regulates protein synthesis and allows progression from the G1 to the S phase of the cell cycle. Rather than activation through somatic mutations of the pathway components, aberrant pathway signaling is associated with activation of IGF and/or EGF.⁶⁷

Gene expression studies in human HCC tissue have found dysregulation of this pathway to be important in tumor progression.⁶⁷ EGF was upregulated, particularly in advanced HCC, and the tumor suppressor PTEN was downregulated in advanced HCC. Also, a subgroup of patients had very high upregulation of IGF-2. In addition, there is a clear shift of mTOR localization in cirrhotic tissue and HCC. In cirrhosis, staining of mTOR is predominantly membranous, whereas in HCC it is typically located in the cytoplasm. Lastly, mTOR positivity is a negative prognostic factor in HCC, with these patients having increased tumor multinodularity and decreased time to recurrence after treatment.

Blocking the mTOR cascade is thought to be a therapeutic option in HCC. mTOR inhibitors, such as sirolimus or everolimus, may decrease tumor growth and expand survival. Potentially combining these drugs with epidermal growth factor receptor (EGFR) inhibitors may allow blockade of the signaling pathway at many levels, which could potentially offer synergy in treatments. Such has been shown in mouse models to be effective *in vivo*, and has been confirmed in small trials in humans.⁶⁸ Larger human trials are under way.⁶⁷ In addition, the combination of sorafenib to block the Ras/Raf pathway, and sirolimus to block the mTOR pathway, may be another effective way to treat liver cancer.^{51,69} mTOR may also interact with other pathways in complex ways. Recently it was found that mTOR inhibition may result in MAPK activation⁷⁰; this suggests that tumors are finding “escape mechanisms” for pathway blockades, which may form the mechanism for tumor resistance to treatments. The understanding of the pathway interactions may allow planning for treatment regimens with combination therapies similar to treatment of resistant-prone viruses.

IGF

IGF is increasingly being recognized as playing a key role in liver cancer signaling. IGF has a major role in fetal development, cell proliferation, differentiation, growth, and apoptosis, and has been implicated in multiple malignancies. IGF-1 stimulates growth through cellular proliferation and inhibition of apoptosis.⁷¹ IGF-2 is overexpressed up to tenfold above normal in 20% to 30% of human liver cancers.⁶ IGFR-2 receptor is a tumor suppressor, and is downregulated in a subset of liver cancers.

The importance of IGF-1 and IGF-2 is highlighted by the rising incidence of liver cancer in patients with insulin resistance, diabetes mellitus, and nonalcoholic steatohepatitis,⁷² as discussed in detail in the article by Siddique and Kowdley elsewhere in this issue. Hyperinsulinemia upregulates the production of IGF-1 and activated insulin receptor substrate (IRS)-1, which is upregulated in liver cancer and is involved in cytokine signaling pathways. In addition, IGF-1 activates the Akt/mTOR pathway which, as discussed earlier, is important in liver cancer.⁷³

EGFR

EGFR is an active target for several oncogenic chemotherapeutics. It may be attacked extracellularly through the use of antibodies that block its receptor, or intracellularly with binding of the receptor's tyrosine kinase domain.⁷⁴ Targeting EGFR has been used in many solid tumor treatments, including cancer of the colon and rectum, head and neck, lung, and pancreas. EGFR is overexpressed in 40% to 70% of liver cancers, and activation of the receptor has been implicated in pathogenesis of HCC.^{75,76} EGFR activation results in signaling through both the Ras pathway and mTOR pathways already discussed, and is an important targeting receptor in HCC.

Angiogenesis/VEGF Pathway

HCC is a highly vascular tumor, and development of new blood vessels is key to allowing progression of disease. Avascular tumors only grow to a certain size and then undergo regression if their metabolic demands are not met. Angiogenesis depends on activation, proliferation, and migration of endothelial cells to create a functional system of blood vessels.⁷⁷ The main factors that stimulate angiogenesis are hypoxia and inflammation. Hypoxia induces expression of hypoxia-inducible factor 1 (HIF1) and IGF-2, both of which stimulate expression of VEGF and other growth factors such as FGF, hepatocyte growth factor, and TGF.⁷⁸ Endothelial cells proliferate in response to growth factors secreted by themselves (autocrine stimulation) or by surrounding cells (paracrine stimulation). In the development of pathologic angiogenesis such as happens in tumor formation in the liver, capillarized vascular structures develop instead of the sinusoidal vascular pattern that occurs in normal tissue. This process hampers the physiologic interchange of oxygen and metabolic factors, which further stimulates hypoxia and tissue injury. It also impairs the effective delivery of therapeutic drugs to the tumor.⁷⁹ Therefore, development of pharmaceutical interventions may be directed at inhibiting progression of angiogenesis as well as facilitating the transcellular route of delivery.

The importance of angiogenesis in tumor development is highlighted by the knowledge that VEGF levels correlate with vascular invasion and metastasis.^{80,81} In addition, FGF levels seem to correlate with advanced tumor stage, vascular invasion, and post-operative relapse.⁸² In patients undergoing transarterial chemoembolization (TACE), VEGF levels also correlate with response rate.⁸³ Patients who have VEGF levels above the mean have a high rate of nonresponse to TACE, with residual tumor activity of greater than 30%. In addition, it has been found that VEGF levels significantly increase in the 24 hours after TACE and decrease after 48 to 72 hours. Therefore, VEGF could be used in monitoring response to treatment, and potentially could be a target for adjuvant treatments such as sorafenib, in patients undergoing transarterial therapy when used as a neoadjuvant medication.

TGF- β

TGF- β is a cytokine that controls many aspects of cell biology, including proliferation, migration, adhesion, differentiation, and modification of the cellular microenvironment.⁷ Genetic alterations of TGF- β have been reported in cancer, but the implications are complex, as TGF- β can exhibit both tumor-suppressive and oncogenic properties.⁸⁴⁻⁸⁶ TGF- β acts as a tumor suppressor at the early stages of tumor development by inhibiting proliferation and inducing apoptosis, but as carcinogenesis progresses TGF- β action changes and can contribute to tumor progression. When cell lines exhibit the late TGF- β signature, they possess a more invasive phenotype and have worse prognosis with a shorter patient survivals.⁷

Because TGF- β activity changes as cancer progresses, it may be possible to develop a gene-expression signature that can help offer prognosis and staging for patients with liver cancer.

FUTURE THERAPIES

In understanding the complex molecular and genetic changes that occur in hepatocellular carcinoma development and progression, it may help to develop future therapies for HCC. At diagnosis, most patients are not candidates for curative therapies, and would benefit from individualized analysis of their tumor genotype and phenotype. With further research, one can envision using therapy that targets an individual's specific tumor type by focusing narrowly on the dominant or codominant tumor-signaling pathway. The hope is that combination therapy will provide greater survival and potentially long-term remission. Thus, new drugs need to be developed that target the single or multiply-activated pathways. Safely performed liver and tumor biopsies can identify a patient's particular tumor fingerprint. Pathways may be blocked vertically, and at more than one location in a particular pathway in this future scenario, which may lead to high-level blockade and prevent tumor resistance via use of alternative "escape" pathways. By contrast, horizontal blockade refers to the use of different drugs targeting different parallel pathways, which may limit the tumor cell's ability to develop resistance to therapy. Horizontal blockade may also limit overlapping toxicities. All of these treatment strategies need to be investigated further to determine the best efficacy and least toxicity of the regimens.

In this article the authors review in detail the molecular and genomic changes that occur in liver cancer. This topic remains a complex one, and discoveries thus far have only scratched the surface. Understanding the molecular and genomic changes that are active in HCC has already allowed targeted therapies to be developed and studied, and will further advance the treatment of liver cancer, allowing for more personalized medicine that may translate into increased survival for patients.

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