2 Molecular Mechanisms of Hepatocellular Carcinoma

Insights to Therapy

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1. INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is increasing in the United States *(1)* and elsewhere *(2)*. Because of its late presentation, its aggressiveness, and its limited response to therapy, HCC is a major cause of cancer death with possibly up to 1 million deaths yearly attributed to HCC worldwide *(3)*. Current treatment modalities for HCC are only modestly successful with orthotopic liver

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transplantation or resection offering the best hope for long-term survival in select patients *(3)*.

Human HCC is commonly associated with underlying chronic liver disease and cirrhosis caused by persistent infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), alcohol abuse, or certain metabolic diseases including hereditary hemochromatosis or α-1-antitrypsin deficiency *(4)*. Although each of these disease processes appears to increase the risk of subsequent HCC development, neither the exact causative insult(s) nor the overall risk posed by these diseases is clearly defined. In general terms, increased hepatocyte replication accompanied by DNA damage and outgrowth of clonal cell populations appears to underlie hepatocarcinogenesis caused by known HCC risk factors. However, it is unclear whether the DNA damage that accompanies the increased mitotic rate is the result of replication errors imparted by abnormal and rapid progression through the cell cycle and/or owing to mutagenesis of the hepatocyte genome directly by toxins or through oxidative stress induced by inflammation or other mechanisms *(5)*. The question of whether similar mechanisms of hepatocyte transformation are shared among the various predisposing conditions or whether unique pathways are employed is under intense scrutiny.

To this end, substantial efforts have been made to understand the genetic basis of HCC, and multiple avenues of research are now converging to offer insight into the molecular mechanisms of liver cancer. It is proposed that five to six separate genetic events are necessary for transformation of a normal hepatocyte into a malignant cell *(6)*, and the use of animal models of HCC, as well as analysis of human HCC tissue samples, has yielded important clues about the molecular steps that lead to the development of HCC. Although mismatch repair mechanisms may play a role in some human HCCs *(7)*, genomic instability characterized by repeated losses and gains of particular chromosomal regions in HCC cells occurs more frequently. Nonrandom losses of heterozygosity (LOH) have been noted on chromosomes 1p, 4q, 6q, 8p, 9p, 10q, 13q, 16p, 16q, and 17p in HCCs, whereas gains of genomic material were identified on chromosomes 1q, 6p, 8q, and 17q *(8)*. The regions of loss or gain are thought to harbor tumor suppressor genes and oncogenes, respectively, and in some instances, these changes correlate with underlying disease condition, tumor differentiation, or patient outcome. Identification of the potential genes lying within the regions has been the focus of many studies, and from them and other experimental evidence such as that obtained from gene expression profiling using cDNA/oligonucleotide microarrays or serial analysis of gene expression (SAGE), some recurrent themes are emerging that may direct the development of novel therapies. Aberrant signaling via cell surface receptors and intracellular effector

molecules, deregulation of the cell cycle and apoptosis, extracellular matrix remodeling, and induction of vascular remodeling and growth all appear to contribute to the neoplastic transformation, growth, and/or subsequent invasion of hepatocellular carcinoma. These pathways in human HCC are highlighted here.

2. HEPATIC MITOGENS IN HCC

Most peptide growth factors bind to and activate cell growth, motility. and survival pathways through cell surface tyrosine kinase-bearing receptors. Their importance to hepatic homeostasis has been a focus of study over the last quarter century, and several growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), and the EGF-related protein, transforming growth factor (TGF)-α are likely to be important in vivo regulators of hepatocyte growth *(9)*. Other modulators such as insulin-like growth factor (IGF)-I and IGF-II have been demonstrated to stimulate hepatocyte DNA synthesis in vitro *(10)*.

2.1. Hepatocyte Growth Factor

In cultured hepatocytes, HGF induces motility *(11)*, causes the adoption of complex hepatic architecture *(12)*, and acts as an anti-apoptotic agent *(13,14)*. It is also the most potent known mitogen for hepatocytes in culture and is thought to be one of the key stimulants of hepatocyte replication in vivo following surgical removal of the liver *(9)*. The biological actions of HGF are mediated through the receptor tyrosine kinase, Met *(15)*. The importance of HGF and Met in liver biology is highlighted by the fact that animals null for HGF or Met die *in utero* with liver, placental, and other abnormalities *(16–18)*.

Several studies show that Met expression is upregulated in human HCC tissues *(19–21)* and, when abundantly overexpressed, may correlate with the presence of intrahepatic metastases and poor patient outcome *(20)*. Activating mutations of the *met* gene *(22)* and gains of chromosome 7 or 7q (or portions thereof) *(23,24)*, where both HGF and Met reside (7q21.1 and 7q31.2, respectively), have been occasionally detected in human liver tumors. HGF expression in human HCC is not consistently upregulated *(21,25)*; however, in vivo experimental models of HGF production in mouse hepatocytes demonstrate that HGF has HCC promoting activity in an autocrine manner in transgenic mice *(26)*.

2.2. EGF and TGF-α

Both EGF and the closely related molecule TGF-α bind to and activate the EGF receptor (EGFR), a tyrosine kinase-bearing transmembrane pro-

tein *(27)*. Like HGF, EGF also stimulates hepatocyte motlility *(11)*, and EGF and TGF-α induce morphogenic changes in hepatocytes *(12)*. This growth factor pair is postulated to provide growth signals to hepatocytes as well as to other hepatic constituents during the regenerative process *(9)*.

Studies have demonstrated enhanced EGF mRNA and/or protein expression in regenerative hepatic nodules *(28)* and in HCCs *(29)*, but analysis of six human HCC cell lines revealed only very low expression of EGF by tumor cells *(30)*. Much data, however, implicate TGF-α in HCC. Mice overexpressing TGF-α in the liver develop HCC after 12 months of age *(31,32)*. Collectively, more than 55% (54 of 94) of human HCCs stained strongly for TGF-α protein as compared to nontumorous adjacent tissue *(33–35)*. TGF-α mRNA abundance was also elevated in HCC tissues and was correlated with HBV infection *(36)*. Hsia et al. *(33)* observed a similar correlation with TGF-α protein and HBV infection. Contradictory data regarding EGFR levels in human HCCs exist, however; levels were reportedly increased in some studies *(37,38)* but unchanged in others *(39,40)*.

2.3. Insulin-Like Growth Factors

The growth and pro-survival substances known as IGF-I and IGF-II have been implicated in hepatic tumor development; as mentioned, they can effect hepatocyte DNA synthesis in cultured cells *(10)*. The IGFs are secreted peptide factors whose relative extracellular concentrations and activities are determined by their interaction with insulin growth factor-binding proteins (IGFBPs) *(41)*. The IGFs signal primarily through the tyrosine kinase containing IGF-I receptor (IGFIR) *(42)*. Tyrosine kinase activity stimulated by IGFs is in part propagated by insulin receptor substrates (IRS-1 through IRS-4) *(43)*.

The human IGF-II gene is genomically imprinted with expression proceeding from only one allele (the paternal allele) in adult tissues *(44)*, except for the liver where biallelic expression is seen *(45)*. The reappearance of monoallelic IGF-II gene expression *(46,47)* with IGF-II fetal-type promoter usage and production of fetal-type transcripts *(48,49)* in human HCCs suggests that IGF-II gene regulation is aberrant in hepatic tumors. Enhanced IGF-II protein and mRNA expression was also detected in human HCCs *(48,49)* and appeared to positively correlate with HBV status *(50)*.

Another mechanism to regulate the function of IGFs in human HCCs may involve altering the relative abundance of IGF-binding proteins; for example, reduced mRNA levels for IGFBPs (IGFBP-1, -3, and -4) *(51–53)* have been detected in human HCCs. Because of the dual effects some IGFBPs like IGFBP3 may have on IGFs *(41)*, it is unclear whether reduced

IGFBP expression in HCCs potentiates or inhibits IGF activity. However, IGFBP3 is a particularly attractive target to be downregulated in tumors given its ability to inhibit IGF-mediated survival as well as inhibit cell growth in an IGF-independent manner. To this end, reduced plasma levels of IGFBP3 are generally associated with an increased risk of some cancers *(42)*.

IRSs are intracellular proteins that become tyrosine phosphorylated after associating with stimulated receptors such as IGFIR; they then couple with effector molecules to activate the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinases (PI3K) pathways to promote cell survival mechanisms *(43)*. In the liver, IRS-1 is phosphorylated during liver regeneration in the rat *(54)*, and 3-month-old mice overexpressing human IRS-1 in the liver under the direction of the albumin promoter showed enhanced hepatocyte DNA synthesis but no tumor formation *(55)*. Regarding human liver tumors, the human homologue of IRS-1 was originally cloned from human hepatocellular carcinoma cells and showed upregulated mRNA expression in HCC tumor tissues as compared to adjacent liver *(56)*.

3. THE PI3K–AKT/PKB PATHWAY IN HCC

PI3Ks comprise a large family of lipid kinases that phosphorylate the inositol moiety in phosphoinositides (PI). Class Ia PI3K signaling stimulated by interaction with tyrosine kinases launches pro-survival, -proliferation, -growth, -motility and -metabolic programs in cells. PI3Ks in this subclass consist of heterodimers containing one of three p110 enzymatic subunits (α, β, or δ) which is regulated by a p85 subunit (α or β); PI3K can also associate with ras *(57)*. Activation of PI3K in cells causes phosphorylation of the D3 position in the inositol ring of PI(4,5)P_2 leading to a rise in PI(3,4,5)P_3 levels, which in turn recruits and stimulates Akt/protein kinase B (Akt/PKB), a serine/threonine kinase that is responsible for amplifying and specifying signals from PI3K *(58)*. For full enzymatic activation of Akt/PKB, phosphorylation by protein-dependent kinase (PDK) is required *(59)*. Phosphatidylinositide phosphatases (PIPases) such as phosphatase and tensin homolog (PTEN) and SH2-containing inositol 5'-phosphatases (SHIPs) control the level of PI(3,4,5)P_3 generated by PI3K *(60)*. PTEN is a lipid and protein phosphatase that reduces the amount of PI(3,4,5)P_3 by dephosphorylating this phospholipid at the 3' position *(61)*. SHIPs are also lipid phosphatases, but they convert PI(3,4,5)P_3 to PI(3,4)P_2 *(60)*.

Studies demonstrate that the PI3K-Akt/PKB pathway becomes activated in normal cultured hepatocytes and liver under various conditions

such as in response to growth factor stimulation *(62)* and during liver regeneration following partial hepatectomy *(63)*, respectively. Interestingly, mice that have been engineered to produce no p85 α-species die perinatally of liver necrosis and other findings *(64)*. In human HCC, a role for the PI3K–Akt/PKB pathway in tumor growth and survival is supported *(65)*. For example, mutation of the PTEN gene, the major PIPase that downregulates the levels of PI3K-generated phospholipids, has been found in some human HCCs *(66)*, whereas analysis of 60 human HCCs and paired nontumorous liver tissues demonstrated diminished PTEN mRNA levels in the malignant component of most cases as determined by Northern blot *(67)*. In other studies, more than 40% (43 of 105) of human HCCs were found to have reduced or absent levels of PTEN protein by immunohistochemistry as compared to adjacent liver tissue. Reduced PTEN staining correlated with higher tumor grade, poorer survival, and increased recurrence *(68)*. Similarly, mice with targeted disruption of one PTEN allele developed tumors in the liver and other organs *(69)*.

4. WNT/β-CATENIN SIGNALING IN HCC

The Wnt/β-catenin signaling pathway is critical to proper embryonic axis development and organogenesis *(70)*. In the adult rat liver, partial hepatectomy stimulates β-catenin nuclear translocation *(71)* suggesting that it regulates physiologic hepatocyte growth. β-Catenin is a multifunctional protein that complexes with a seemingly diverse array of proteins including the cell adhesion molecule, E-cadherin; several proteins involved in β-catenin degradation consisting of axin, glycogen synthase kinase-3 β, protein phosphatase 2A, and adenomatous polyposis coli (APC) protein; transcription factors including lymphoid-enhancing factor (LEF)/T-cell factor (Tcf) *(72)*; the Met tyrosine kinase receptor *(73)*, and others.

The amount of unbound free β-catenin in the cytosol is tightly regulated by its degradation by GSK-3 β through serine/threonine phosphorylation of β-catenin's N-terminal domain *(72)*. Wnt protein, when present, binds to its cell surface receptor frizzled and, via dishevelled, GSK3β-mediated phosphorylation of β-catenin is inhibited. Thus, β-catenin protein dissociates from the axin-containing complex, accumulates in the cytoplasm, and then translocates to the nucleus *(70)*. There, it associates with LEF/Tcf to stimulate transcription of target genes such as *c-myc* (74) and cyclin D1 *(75)*.

Several mechanisms that lead to the reactivation of the Wnt/β-catenin-signaling pathway in human HCC have been proposed. The first is through mutation of the β-catenin gene in exons encoding for the GSK-

3β phosphorylation sites, especially exon 3. This is seen in up to 44% of human HCCs examined by single-strand conformational polymorphism (SSCP) *(76,77)*; however, other estimates of the β-catenin mutation rate place it at about 22% on average in human HCCs *(8)*. Interestingly, a study of 25 dysplastic hepatocellular nodules showed no detectable mutations in the β-catenin gene nor enhanced β-catenin cytoplasmic or nuclear staining *(78)*. β-Catenin gene mutation is particularly common in HCCs from HCV-infected patients *(76)* and, in a separate study, from non-HBV-positive patients *(77)*; it is also associated with a favorable prognosis *(77)*. However, examination of 23 HCC tumors from Malaysian patients showed no β-catenin mutations suggesting that the β-catenin gene may be differentially targeted for mutation depending on the underlying HCC risk factor(s) and/or genetic composition of the population *(79)*.

Nuclear accumulation of β-catenin protein has also been identified in the absence of β-catenin gene mutation. Although the APC gene is frequently lost or mutated in solid tumors such as colon carcinoma and has been linked to abnormal accumulation of β-catenin, APC is not considered to be a major target in HCCs *(80)*; however, Piao et al. *(81)* noted that 20% of human HCC cases had LOH of the APC gene. Interestingly, a case report has documented a patient harboring a germline mutation for APC as the only risk factor for development of HCC. The tumor showed somatic mutation of the remaining APC allele suggesting that patients who inherit an APC mutation may be at risk of developing HCC *(82)*. Because APC may not be a common target for mutation in human HCC, axin has been evaluated. Studies of human HCC have shown that the chromosome arm that harbors the axin1 gene (16p) often displays LOH, although mutation (consisting of point mutations, small deletions, or small insertions) of the axin1 allele has been identified in less than 10% of cases *(83,84)*.

Augmentation of the Wnt/β-catenin-signaling pathway may occur through modifying β-catenin's transactivating properties. To this end, a scaffolding protein known as EBP50 was recently shown to associate with β-catenin and enhance β-catenin's transcriptional activity in in vitro assays. Immunohistochemical staining revealed that EBP50 was overexpressed in 21 of 38 (55%) human HCC tumors as compared to adjacent nontumorous tissues and that increased EBP50 protein accumulation correlated positively with nuclear β-catenin immunostaining (85) suggesting that EBP50 and β-catenin could cooperate in promoting liver tumorigenesis.

Another mechanism for abnormal intracellular accumulation of β-catenin protein has been proposed by Cui et al. (76) who described an

enhanced *in situ* hybridization signal for β-catenin mRNA in the cytoplasm of some human HCCs lacking β-catenin gene mutations in exon 3. They suggest that translational activity of abnormally high levels of β-catenin mRNA in HCCs results in an overabundance of β-catenin protein, which ultimately overwhelms the GSK-3β ubiquitination pathway and promotes β-catenin accumulation.

Reactivation of the Wnt/β-catenin-signaling pathway may also occur through downregulation of E-cadherin. Human HCCs commonly show allelic imbalance on chromosome 16q, particularly in the vicinity of the E-cadherin gene (16q22.1) *(86,87)*, and downregulation of E-cadherin protein expression has been seen in HCCs as well *(87,88)*. Although no mutations of the E-cadherin gene have been reported, CpG methylation of the E-cadherin promoter in HCC *(89)*, increased expression of a putative transcriptional repressor (i.e., Snail) of the E-cadherin gene in HCC cell lines *(90)* and polymorphic differences in E-cadherin-promoter nucleotides *(91)* as possible means of downregulating its gene expression have been postulated.

Currently available transgenic mouse models of β-catenin expression in the liver unfortunately do not clarify the role of β-catenin in hepatic tumorigenesis. Studies have demonstrated that stabilized forms of β-catenin either lacking the N-terminus including the GSK-3β phosphorylation and the α-catenin-binding sites *(92)* or engineered to undergo deletion of the third exon containing the GSK-3β phoshorylation sites through cre-mediated recombination *(93)* are associated with hepatomegaly related to increased hepatocyte proliferation in one model *(92)* and mitochondrial dysfunction in the other *(93)* as compared to control animals. Because of enhanced morbidity and limited survival of either set of transgenic animals (3 to 4 weeks for those with N-terminal deletion of β-catenin and more than 6 months for the animals undergoing adenovirus-mediated cre recombination), the mice could not be fully evaluated for the development of hepatic tumors *(92,93)*; however, Harada et al. *(93)* speculate, based on their findings in mice expressing a dominant form of β-catenin lacking the GSK-3β phosphorylation sites via deletion of exon 3, that stabilized β-catenin expression alone is insufficient to induce liver tumors. These results will require further in vivo evaluation.

5. THE RAS SUPERFAMILY IN HCC

The superfamily of small GTP-binding proteins including ras and rho family members is involved in regulation of normal cell proliferation by controlling the expression and activities of regulatory molecules such as cyclin D1, p21Waf1/Cip1, and p27Kip1 following exposure to mitogens

or other stimuli *(94)*. Regarding normal liver, ras is involved in hepatocyte replication under in vitro culturing conditions *(95)* as well as during regeneration following partial hepatectomy in rodents *(96,97)*.

Three closely related members, H-, K- and N-*ras*, make up the immediate family of ras molecules and are all commonly targeted in human cancer. Of the three members, the gene for K-*ras* is more often found to be mutated than the others *(98)*. Humans occupationally exposed to vinyl chloride may develop HCC as a consequence *(99)*, and K-*ras* mutation may be an integral step in the process. HCC tumors from individuals exposed to vinyl chloride were examined for K-*ras* mutation, and 33% (6 of 18) of cases were found to be positive. In two of six cases of suspected vinyl chloride-induced HCC that harbored K-*ras* mutation, adjacent liver tissue showed K-*ras* mutation as well. HCCs from patients with other known etiologies (viral infection or ethanol) showed a K-*ras* mutation rate of 15% in this study *(100)*. However, other researchers have rarely detected K-*ras* mutations in human HCC *(101,102)*.

Mutation of the H-ras gene, like the K-*ras* gene, is uncommonly detected in human HCCs *(102,103)*; surprisingly, however, LOH in the vicinity of the H-ras locus on the short arm of chromosome 11 (11p15.5) was found to be prevalent in one study of human HCC, seen in about 42% of cases by Southern blot analysis *(103)*. Others have shown that the intensity of immunostaining for ras in human HCCs appears to be diminished in more poorly differentiated lesions as compared to cirrhotic liver or well-differentiated tumors *(104,105)*, suggesting that ras plays a role in the early stages of carcinogenesis.

Deletions of human chromosome 8p, particularly at 8p21.3-22 *(106)*, are frequently identified in HCC and are associated with the presence of metastatic hepatocellular carcinoma *(107)*, suggesting that a tumor-suppressor gene involved in aggressive HCC behavior resides in the region. To this end, cloning of a possible target gene in the region 8p21.3-22 named deleted in liver cancer-1 (DLC-1) was carried out *(108)*. DLC-1 is related to the rat p122 RhoGAP gene, the product of which negatively regulates the activity of rho. Approximately 50% of human HCCs examined showed loss of heterozygosity of the DLC-1 gene *(108)*, whereas in other studies, 20 to 67% of cases lacked mRNA expression of DLC-1 in tumors *(109,110)*. Enhanced methylation at a CpG island 5' to the DLC-1 gene, which may account for reduced DLC-1 gene transcription, was found in 24% (6 of 25) of HCC cases as compared to adjacent liver tissues *(110)*. A homolog to DLC-1, deleted in liver cancer-2 (DLC-2), has been identified. It likewise has signatures of RhoGAP. The gene for DLC-2 is localized to 13q12.3 in humans, a site commonly found to undergo LOH in HCC *(111)*. More than 35% of informative human HCC cases showed

LOH for two markers flanking DLC-2, whereas reverse transcriptase polymerase chain reaction carried out on those samples showing LOH demonstrated a reduction in DLC-2 mRNA levels in about 18% of tumors compared to adjacent liver tissues *(112)*. Recently, gene expression profiling of human HCCs revealed that the expression of a gene for a small GTPase known as ARHC (RhoC) and a gene for a putative small GTPase-regulating protein known as ARHGAP8 (RhoGAP8) were preferentially up- and downregulated, respectively, in a survey of invasive HCCs as compared to noninvasive tumors *(52)* suggesting that vascular invasion of HCC cells may involve the uncontrolled activity of Rho proteins.

6. C-MYC IN HCC

The myc family of nuclear proteins, to which c-myc belongs, has a wide repertoire of biological functions including growth control, apoptosis, and differentiation, and when deregulated, tumorigenesis. How the members carry out their routine functions is complex and may involve regulation of histone acetylation/deacetylation in the promoter regions of target genes as well as sequestration and inhibition of transcriptional activators *(113)*. Through these interactions, it is proposed that myc, under normal circumstances, protects cells from unregulated growth by simultaneously promoting proliferation while sensitizing cells to apoptosis without directly activating the apoptotic cascade *(114)*.

In the liver, *c-myc* gene expression has been extensively studied in in vitro and in vivo models. Rat HCCs induced by various carcinogenic regimens often show amplification of the *c-myc* gene *(115,116)* and, when *c-myc* was overexpressed in the livers of transgenic mice under an inducible promoter, HCCs resulted *(117)*. In the woodchuck model of HCC, which is induced by infection with woodchuck hepatitis virus (WHV), integration of WHV DNA into the woodchuck genome is often seen in the vicinity of the *N-myc (118)* and *c-myc (119)* genes resulting in their gene activation.

In humans, a fairly robust percentage of HCCs demonstrate gain of genetic material on chromosome 8q by comparative genomic hybridization (CGH) *(8)*. An increase in gene copy number for *c-myc*, which resides at 8q24 was seen cumulatively in about 39% (24 of 62) of human HCCs, and was associated with a poor prognosis and with moderately and poorly differentiated tumors *(120, 121)*. Analysis of *c-myc* mRNA and protein levels demonstrated a progressive increase from normal liver, nontumorous liver, cirrhotic liver, well-differentiated HCC to poorly differentiated HCC *(122)*, but another study did not observe this pattern *(123)*.

7. CELL CYCLE REGULATORS IN HCC

Progression through the cell cycle is a highly orchestrated event employing numerous regulatory proteins. Some of the most well known of these proteins, such as p53 and Rb, are also clearly involved in tumorigenesis of various organs. Cyclin-dependent kinases (cdks), cyclins and cdk inhibitors such as p27 Kip1, p21Waf1/Cip1, and p16INK4A drive the cell cycle through phosphorylation and/or degradation of key substrates *(124)*. As the identities and functions of other cell cycle regulators are revealed, it is becoming obvious that they too can participate in tumor development.

7.1. p53

In human HCC, the short arm of chromosome 17 is perhaps the most frequent chromosome targeted for deletion *(6)*, particularly at 17p13, which harbors the gene for the tumor suppressor molecule p53. Under normal circumstances, cell cycle arrest or apoptosis can result from p53 activation following cellular stress, and this is owing in part to p53-mediated transcriptional regulation of target genes such as p21Waf1/Cip1, 14-3-3σ and Bax *(125)*. Loss of heterozygosity at the p53 locus was seen in 57% (8 of 14) of human HCCs in one study, and more than half (63%) of the cases displaying p53 LOH in tumors showed allelic loss of p53 in adjacent liver tissues as well *(126)*. Mutation of the p53 gene occurs with some regularity in HCCs and is associated with higher grade lesions *(127)*, vascular invasion *(128)*, and lower survival rate *(129)*, although the latter is under question *(130)*. In human HCCs worldwide, p53 gene mutation has been reported to occur in about 28% of cases on average *(8)*, but this incidence increases in certain geographic areas. In patients from regions of China and Africa, HCCs harbor p53 mutations in an estimated 55% of cases with specific mutation at codon 249 (G to T transversion) of p53, accounting for up to 82% of all p53 mutations in these populations *(6)*. It is suspected that aflatoxin, a carcinogenic compound produced by certain members of the *Aspergillus* genus of fungi, contaminates food grains from these regions and induces DNA adducts in hepatocyte DNA following consumption. Codon 249 of the p53 gene is believed to be particularly susceptible to aflatoxin-induced mutagenesis *(6,131)*. HBV infection, which is highly prevalent in these geographic areas, may synergize with aflatoxin to promote hepatocarcinogenesis *(132)*. To this end, Hbx, an HBV-encoded protein, has been shown to bind to and inhibit the activity of p53 *(133,134)* and DNA repair proteins *(135)*, which may allow the DNA of infected hepatocytes to accumulate mutations *(6)*, but this remains speculative.

Although mutation in the coding region of the p53 gene is not uncommon, other rare mechanisms for inactivating p53 such as promoter methylation *(136)* and mutation of the p53 gene at the intron-exon boundary *(137)* have been reported. Altered activity or expression of p53-modulating proteins is another mechanism to affect wild-type p53 function. As mentioned, the viral protein Hbx can reduce p53 action through physical association; murine double minute clone 2 (mdm-2) protein is another candidate. It binds to p53 masking its transactivation domain and targets it for degradation; mdm-2 also associates with other proteins such as Rb and E2F1 and may modulate gene expression *(125)*. The abundances of mdm-2 mRNA *(138)* and protein *(139)* are noted to be increased in about a 25 to 50% of human HCC cases, and increased mdm-2 expression appears to correlate with reduced survival. Interestingly, the abundance of mdm-2 mRNA in tumor tissues as compared to adjacent nontumorous tissues was found to be particularly elevated in tumors lacking p53 gene mutation at codon 249 as compared to those with p53 gene mutation at this location, supporting the hypothesis that mdm-2 is upregulated to inhibit wild-type p53 in tumors lacking mutant p53 *(138)*.

7.2. Rb

Allelic imbalance is observed on chromosome 13q in about 30% of human HCCs *(8)*. Because the Rb tumor suppressor gene (13q14) resides on this chromosomal arm, much attention has been focused on whether Rb participates in HCC development. Rb regulates cell cycle progression into *S*-phase following growth stimulation, is linked to apoptosis induction through a p53-dependent pathway, and is a common target in cancer development *(140)*. Two studies show that 42 to 73% of human HCCs harbor specific loss of one Rb allele *(141,142)*. This was notably accompanied by LOH of Rb in surrounding cirrhotic tissues in about 70% (8 of 11) of HCC cases *(142)*. Identification of genetic alterations in cirrhotic tissues is supported by others: Roncalli et al. *(143)* identified losses of chromosome 13q, as well as 1p, 4q, and 18q, in the cirrhotic livers of patients who later went on to develop HCC, demonstrating that hepatocytes in cirrhotic nodules already take on clonal characteristics and suggesting that the involved chromosomal regions harbor genes important to the early stages of neoplastic transformation.

Given the prevalence of LOH at the Rb locus, mechanisms to inactivate the other allele have been identified. Mutation (in the form of small deletions) of the second Rb allele has rarely been detected in human HCCs displaying LOH for Rb *(141)*. Mutation of the Rb gene promoter may be an unlikely contributor to loss of Rb expression in HCCs *(144)*, but a recent study suggests that 24 of 100 (24%) human HCCs of differing

etiology (HCV, HBV, or alcohol-induced) harbored aberrant Rb gene-promoter methylation *(145)*. Other mechanisms, such as increased degradation of Rb protein, have been proposed as means of reducing Rb levels in tumor cells, and one protein, gankyrin, an oncogenic molecule that induces Rb phosphorylation and speeds its destruction through the ubiquitin–proteasome pathway, may play such a role. This is particularly true in HCC, where gankyrin is reportedly overexpressed in all cases studied *(146)*. Recently, this finding was confirmed; 97% (62 of 64) of human HCC samples showed moderately to markedly increased gankyrin mRNA abundance as compared to adjacent nontumorous liver tissues using Northern blot analysis *(147)*.

Although Rb protein loss is observed in about one-fourth of human HCCs (collectively 27 of 102 cases *[141,148]*), an increase in its abundance in liver tumor tissues has been noted with nearly equal frequency (ranging from 18 to 58% of tumors *[148,149]*). Hui et al. *(148)* found that alterations in Rb levels in human HCCs, regardless of whether increased or lost, were associated with later tumor stages or with the presence of HCC metastases.

Rb controls *S*-phase entry by associating with the E2F family of transcription factors such as E2F1. This interaction is mediated in a phosphorylation-dependent manner by a complex containing a cdk and a cyclin, particularly the cdk-4/Cyclin D1 complex. Phosphorylated Rb no longer binds to and sequesters E2F1, which is then free to alter transcription of cell cycle-related genes *(140)*. p16INK4A is an inhibitor of cdks such as cdk-4 and as such indirectly reduces Rb phosphorylation. The p16INK4A gene resides on human chromosome 9p21, an area that is lost in some human HCC cell lines *(150)*. Germ-line mutation of the p16INK4A gene in humans is associated with familial melanoma *(151)*, and in rodent models, gene knockout studies in mice show that biallelic loss of the p16INK4A homologue results in B-cell lymphomas and soft-tissue sarcomas *(152)*. p14ARF/p19ARF, a regulator of mdm-2, shares exons of the p16INK4A gene, and its expression is induced by E2F1, thus linking the Rb and p53 pathways *(153)*. Homozygous deletion of the p16INK4A locus could therefore interfere with both Rb- (via cdk-4-mediated inactivation) and p53- (via mdm-2-mediated inhibition of p53) dependent mechanisms.

Evaluation of p16INK4A in human HCCs has revealed an absence of functional p16INK4A in up to 70% of cases *(154,155)*. Homozygous deletion of the p16INK4A locus was detected in 60% of human HCCs in one study *(156)*. About 50% of HCCs examined for p16INK4A mRNA and protein levels showed reductions *(155,157)*, with abundances gradually decreasing with increasing tumor stage *(157,158)*. Additional mecha-

nisms to downregulate p16INK4A, such as gene promoter hypermethylation, somatic p16INK4 gene mutation, reduced p16INK4A gene transcription through enhanced Id-1 transcriptional repressor expression, and posttranscriptional mechanisms have been proposed *(145,157,159,160)*. Interestingly, four patients with HCC were found to harbor germ-line mutation of one p16INK4A allele, and in two of the patients, loss of the remaining p16INK4A allele in the tumor was detected, suggesting that those with inherited mutations at the p16INK4A locus are at risk for developing HCC *(159)*.

At least two other cdk inhibitors (CDKIs) p21Waf1/Cip1 and p27Kip1 have been linked to human HCC. p21Waf1/Cip1 is a multifunctional protein that modulates diverse cellular functions including cell cycle progression through direct interactions with cyclins, cdks, and E2F; DNA synthesis by binding to proliferating cell nuclear antigen (PCNA); apoptosis by binding to and inhibiting pro-caspase 3; and cell differentiation *(161)*. Levels of p21Waf1/Cip1 mRNA and protein have been examined in human HCCs. Amounts of p21Waf1/Cip1 mRNA tended to be reduced in tumor tissues as compared to adjacent nontumorous tissues *(162–165)*. p21Waf1/Cip1 protein overabundance, on the other hand, was detected in 33 to 64% of HCCs *(149,165)*.

Expression of the CDKI p27Kip1 *(166)*, a protein that is structurally related to p21Waf1/Cip1 and similarly targets the cdk-2/Cyclin E complex in particular for negative regulation *(167)*, has also been investigated in HCCs. Unlike p21Waf1/Cip1 mRNA levels that seem to be markedly downregulated in HCCs, p27Kip1 mRNA abundance appears to remain roughly unchanged or slightly increased in tumor tissues as compared to adjacent nontumorous tissues *(168)*. However, multiple laboratories *(168–170)* demonstrated reduced p27Kip1 protein levels in some HCCs, particularly those having aggressive features such as higher tumor stage *(168)*, portal invasion, poor differentiation, and large size *(169)*. Moreover, these groups *(168–170)* also independently demonstrated a correlation between reduced tumor staining for p27Kip1 protein and poor patient outcome. That decreased p27Kip1 protein staining in HCCs repeatedly correlated with poor patient outcome is a consistent finding for tumors originating in other tissues such as carcinoma of the breast *(171)*.

Amplification of the long arm of chromosome 11 has been identified in human HCC *(172)*. On this chromosomal arm resides the gene for cyclin D1 (11q13), and up to 18% of HCC cases harbor an increased gene copy number for cyclin D1 *(145,173,174)*. Evaluation of cyclin D1 mRNA or protein expression showed enhanced protein levels in HCC tumors that displayed gene amplification as compared to tumors lacking amplification or normal liver, which both demonstrated weak or negative staining.

The presence of cyclin D1 gene amplification was identified in advanced stage tumors with rapid growth suggesting a role for this cell cycle regulator in promoting aggressive neoplastic behavior in some HCCs *(174)*. It should be noted, however, that reduced cyclin D1 gene expression was observed by two groups analyzing global gene expression in human HCCs by cDNA microarray *(52)* and serial analysis of gene expression *(175)* techniques. Nonetheless, the oncogenic potential of cyclin D1 in the liver has been verified in a transgenic mouse model in which the cyclin D1 gene was placed under the control of the rat liver fatty acid-binding protein promoter directing transgene expression to the liver and intestines. Liver abnormalities characterized by hyperplastic changes at 3 months of age, hepatomegaly and dysplasia by 6 months, and adenomas by 9 months were observed in the transgenic animals culminating in HCC development in 31% of mice by 17 months *(176)*.

8. GROWTH INHIBITORS AND APOPTOSIS MEDIATORS IN HCC

Apoptosis is important to tissue homestasis and morphogenesis, and when deregulated, can contribute to carcinogenesis *(177)*. At least two cellular pathways mediate apoptotic signals: the mitochondrial or intrinsic pathway and the death receptor or extrinsic pathway. Numerous pro- and anti-apoptotic molecules such as bad, bax, and survivin appear to modulate the final outcome *(178)*. Under experimental conditions in vitro and/or in vivo, normal hepatocytes are sensitive to growth inhibition and/or apoptosis induced through activation of the Fas death receptor *(179,180)*, by exposure to TGF-β *(181)*, or via other mechanisms.

8.1 TGF-β

TGF-β is cleaved intracellularly and secreted as a latent molecule. Activation of TGF-β may involve interaction with plasmin, metalloproteinases, or the M6P/IGFII receptor, among other molecules. The signaling receptor system for active TGF-β is a heterodimer consisting of the serine/threonine kinase containing TGF-β receptors I and II (TGF-βRI and II). Intracellular signaling is mediated mostly by Smad proteins (such as Smad2, 3, and 4) which target the nucleus to alter gene transcription of cell cycle regulating and other genes in the capacity of co-activators *(182)*.

Experiments with cultured rodent liver epithelial cells demonstrated that they undergo neoplastic transformation in the presence of TGF-β presumably through selection of TGF-β resistant clones *(183)*, and more than half (59%) of transgenic mice overexpressing TGF-β in the liver

spontaneously develop hepatic tumors by about 16 months of age *(184)*. In humans, HCCs have been shown to overexpress TGF-β *(185)*, and plasma levels of TGF-β in patients with HCC are elevated *(186)*. Expression of TGF-βRI and II mRNA and protein are reduced by 49 and 60%, respectively, in human HCCs as compared to the adjacent tissue *(187)* and mutation of the TGF-βRII *(188,189)* and Smad (Smads2 and 4) genes *(190)* occasionally occurs.

Analysis of the long arm of chromosome 6 (6q) in human HCCs has shown that LOH is a common occurrence and may be associated with a poor prognosis *(84)*. Most work has centered on the region of 6q25-27, which harbors the M6P/IGFIIR, a cell surface protein that promotes activation of latent TGF-β *(191)* and facilitates lysomal degradation of IGF-II *(192)*. Several studies have shown that up to 64% of HCCs demonstrate LOH in this region *(193–195)*, although another study noted no LOH at the M6P/IGFIIR locus in the examined cases *(196)*. Concomitant missense mutations or major amino acid substitutions were identified in the remaining M6P/IGFIIR allele in approx 25% of cases showing 6q25-27 LOH in one study *(197)*. M6P/IGFIIR protein levels were also reduced in about 65% of human HCCs examined *(187)*.

8.2. Fas

The death receptor Fas (CD95) and its ligand, FasL, are well-characterized mediators of apoptosis in a variety of cell types and may also be involved in liver disease *(198)*. Cultured human HCC cells are resistant to Fas-mediated apoptosis *(199)*; however, in human HCCs, Fas, and FasL expression levels are reportedly variable *(200–203)*. Interestingly, Lee et al. *(202)* saw LOH at the Fas locus on human chromosome 10q24.1 in 15% (5 of 34) of informative HCC cases, but no Fas gene mutations were identified.

8.3. Other Apoptosis and Survival Regulators

The expression levels of a variety of other apoptosis regulating molecules have also been examined in human HCCs. Anti-apoptotic molecules such as soluble Fas *(202)*, Fas-associated phosphatase-1 *(202)*, Bcl-xL *(204)*, and survivin *(205)* were found to be expressed at normal or elevated levels in human HCCs, whereas the expression of pro-apoptic molecules such as bcl-2 *(202)*, bid *(206)*, and caspase 3 *(207)* were found to be moderately reduced or absent (as in the case of bcl-2).

Because conventional therapeutic agents often rely on an intact apoptotic mechanism to kill tumor cells *(208)*, deregulated apoptosis in

HCCs may provide one explanation as to why these agents are less than successful in curtailing tumor growth.

9. EXTRACELLULAR PROTEASES IN HCC

The hepatocyte is normally surrounded by and secured to a scant meshwork of extracellular matrix (ECM) proteins consisting mostly of tenascin, fibronectin, and collagen types I, III, and IV *(209,210)*. Integrins anchor hepatocytes and other cells to the ECM *(211)* and regulate cellular functions such as migration, survival, and anoikis by transmitting signals to the nucleus from extracellular cues *(212,213)*. These pathways may be targeted in malignant transformation of cells to promote survival and invasion *(213)*. In the cirrhotic liver and in HCC, the composition of the ECM is altered primarily through the enhanced deposition of collagen type I *(214)* in the former, with addition of collagen type IV *(215)* and laminin *(216)* in the latter; upregulation of the laminin receptor (integrin-α6) on the hepatocyte surface is also seen in dysplasia and carcinoma *(215,216)*.

ECM remodeling appears to be a feature of the liver as it undergoes repair and regeneration following loss of liver mass such as after partial hepatectomy *(210)*. In hepatocarcinogenesis, as in other tumors, degradation/remodeling of the ECM is considered to be an integral step in the development of intrahepatic and distant metastases *(217,218)*. Occasionally, even small HCCs have been shown to metastasize following resection *(219)*, suggesting that some hepatocytes aggressively acquire the necessary repertoire of gene expression to effect growth, invasion, and motility early in neoplastic development *(220)*.

Numerous proteases such as the plasminogen activators (PAs) and matrix metalloproteinsases (MMPs) have been implicated in ECM remodeling in the liver during the regenerative response *(221,222)*. Some of these same proteases may also play a role in growth and invasion of hepatic tumors *(218)*. Urokinase-type plasminogen activator (uPA) and the MMPs, particularly MMP-2 and -9, appear to be involved in both processes. uPA is a serine protease that generates plasmin from plasminogen *(223)* and activates HGF *(224)*. uPA activity is regulated by PA inhibitors (PAI)-1 and PAI-2 *(223)*. Plasmin, meanwhile, can activate MMPs *(225)*, which in turn degrade ECM proteins and activate growth factors, among other functions *(226)*.

The expression of the uPA receptor (uPAR), a protein that promotes uPA activation and may mediate intracellular signals *(223)*, has been shown to be upregulated in human HCCs, and 75% of patients with high uPAR expression in their tumors vs about 15% of those lacking uPAR

tumor expression had HCC recurrence in one study *(227)* suggesting that expression of uPAR by HCCs may be involved in development of recurrent disease. Several investigators have found that MMP-9 mRNA levels are also upregulated in human HCCs *(228–230)*. Giannelli et al. *(231)* surveyed patients with and without metastatic HCC for the expression of MMP-2 and tissue inhibitor of metalloproteinase (TIMP)-2 in primary and/or metastatic HCC tissues as well as patients' sera. They found that, while MMP-2 levels in HCC tissues or serum were not statistically different between those with or without metastases, the levels of TIMP-2 were significantly elevated in tissues and sera of those lacking metastases and correlated positively with survival outcome

10. PRO- AND ANTI-ANGIOGENIC FACTORS IN HCC

Blood vessel formation is essential to the expansion of solid tumors *(232)*. Numerous pro- and anti-angiogenic factors are known, and the interplay between them may be a key component of HCC tumor angiogenesis *(233)*. As HCCs are highly vascular lesions, new blood vessel formation is often exuberant. Early in HCC development the blood supply is often derived from the portal circulation and vascularity is less prominent, but as tumors enlarge and lose their differentiation, the feeding vessels become more pronounced and receive blood from the hepatic artery *(233)*. Two endothelial-specific pro-angiogenic classes of growth factors have been identified: the vascular endothelial growth factor family consisting of six members currently (VEGF-A through E, and placenta growth factor) and the angiopoietins (Ang1 through 4) *(233,234)*. Three endothelial-expressed tyrosine kinase cell surface receptors exist for VEGF including flt-1, KDR/flk-1, and flt-4. The activities of VEGF-A appear to be mediated primarily through KDR/flk-1 *(235)*.

In human HCCs, most studies suggest that VEGF expression is upregulated *(236–239)*, whereas data on the expression of its receptors are less clear. For example, KDR/flk-1 mRNA abundance was upregulated in tumor tissues in one study *(240)*, but Ng et al. *(236)* saw preferential upregulation of Flt-1 mRNA rather than KDR/flk-1 mRNA in their cases of human HCC.

Angiopoietins (Ang) were recently discovered as ligands of the Tie2/Tek vascular endothelium-specific receptor. Although Ang-1 can activate the Tie2/Tek receptor, Ang-2 appears to be an antagonist of Ang-1 and is incapable of inducing Tie2/Tek receptor phosphorylation *(241)*. Enhanced mRNA levels of Ang-2 were noted in HCC tumor tissues and positively correlated with the degree of tumor vascularity as determined presurgically by angiographic studies. Ang-1 mRNA levels were roughly equal between tumorous and nontumorous tissue *(242)*. Others have

shown that Ang-2 protein expression as determined by immunohistochemistry was upregulated in human HCCs as compared to normal liver tissue from patients undergoing liver resection or autopsy for non-liver-related disease and was most highly expressed in poorly differentiated highly vascularized HCCs *(239)*. These findings are consistent with the proposed roles of Angs in angiogenesis in some organs: Ang-2 expression correlates with formation of nascent vessels, whereas that of Ang-1 is associated with blood vessel stabilization *(241)*. Evalulation of Tie2/Tek expression by immunohistochemistry in human HCCs showed that the vascular endothelium present in moderately and poorly differentiated tumors stained more intensely than that of well-differentiated tumors. Tie2/Tek-positive tumors also tended to be larger than Tie2/Tek-negative tumors *(243)*.

Endogenous anti-angiogenic factors have been discovered that inhibit tumor vasculogensis. Interestingly, one protein with anti-angiogenic properties is angiostatin, which is derived from plasminogen by enzymatic cleavage *(244)*. MMP-12/human macrophage metalloelastase (HME) *(245)* is one of several enzymes that cleave plasminogen to produce angiostatin. MMP-12/HME mRNA and angiostatin protein were found to be expressed in more than half of HCC tumor samples. In patients whose tumors were negative for both MMP-12/HME and angiostatin, poorer survival was seen *(246)*.

11. MOLECULAR TARGETS OF HCC THERAPY

If untreated, hepatocellular carcinoma has a dismal prognosis with death usually occurring within 6 months of diagnosis *(3)*. Presently, perhaps the best hope for extended survival in patients with small HCC and cirrhosis is liver transplantation because it may effectively eliminate the tumor(s), the risk of developing metachronous lesions in a cirrhotic liver, and end-stage liver disease all at once *(247)*; however, resection remains a viable option, as well, for select patients *(3)*. For those with advanced disease, current treatment protocols have not been very successful in improving patient outcome *(248)*. Thus, other methods of prevention and therapy are desperately needed.

Because viruses (HBV and/or HCV) commonly underlie the development of human HCC *(3)*, preventing viral infection or curtailing viral replication and progression to cirrhosis are logical long-term solutions, and it has been suggested that serious effort be directed at the former *(249)*. These ideas have been placed into practice with promising results. In Taiwan where HBV is prevalent and perinatal maternal–infant and horizontal childhood transmission are common routes of infection, imple-

mentation of a universal childhood vaccination program for HBV begun in 1984 resulted in a drop in the average annual incidence of and mortality from HCC in children by roughly 50% in 13 years *(250)*.

Unfortunately, vaccines for HCV have not yet been developed. One treatment protocol for those with HCV infection is therapy with interferon (IFN)-α, which is believed to inhibit viral replication and modulate the immune response *(251)*. Initially, IFN-α was used as a monotherapy; however, combination therapy with ribavirin may be superior in achieving biochemical and virological responses *(252)*. Evidence suggests that treatment with IFN-α causes a reduction in progression of liver fibrosis as well as HCC development; however, the effect on HCC has not been noted by all investigators *(251)*. Thus, long-term randomized clinical trials are needed to evaluate the role of IFN in human HCC development.

Administration of acyclic retinoids to patients in the hopes of preventing recurrent and metachronous HCCs following hepatic resection or percutaneous ethanol therapy has been carried out in Japan. Improved survival and a reduction in second primary lesions were seen in patients receiving acyclic retinoids *(253,254)*. Possible mechanisms explaining the inhibitory effect of acyclic retinoids on development of second primary HCCs include induction of apoptosis and differentiation which has been observed in human HCC cells cultured in the presence of these compounds. The effects of acyclic retinoids on tumor cells may be mediated by retinoid X receptor(RXR)-α, a nuclear hormone receptor involved in gene transcriptional regulation, which appears to become aberrantly phosphorylated and inactivated in HCC cells and tissues. Phosphorylation coupled with reduction of endogenous retinoids in HCCs may inhibit RXR-α-mediated transcription resulting in cell proliferation and dedifferentiation, which is overcome by treatment with acyclic retinoids *(255)*.

With the burgeoning of molecular information about human HCCs from numerous research domains, there is hope that current therapies will be improved and new treatments will be discovered. Albeit rare at this point in time, this has clearly been the case with tumors of other organ systems. For example, the fact that many different tumors show activation of the c-kit tyrosine kinase receptor, particularly through mutation, led to the discovery and clinical use of an inhibitor of kit tyrosine kinase, STI571. Therapeutic responses have been seen in some patients with kit-positive tumors such as gastrointestinal stromal tumors (GISTs) after STI571 administration *(256)* and suggests that these types of pharmacogenetic approaches may be avenues for HCC researchers to pursue. To this end, some of the pathways that appear to be involved in HCC development are already the focus of studies by researchers assessing pharmacological and genetic interventions for other tumors.

Although gene therapy protocols for the treatment of human tumors like HCC initially generated great excitement, enthusiasm has been tem-

pered somewhat by waning expression of transgenes, poor transfection efficiency and specificity, and safety concerns *(257)*. Hepatic tumors are also innately difficult to transduce owing to formation of a blood–tumor barrier that blocks vector diffusion to neoplastic cells *(258)*. Because of the various limitations of gene therapy that need to be overcome, targeting key components of pathways important to neoplastic transformation with small molecular inhibitors like STI571 in GISTs *(256)* or human-mouse chimeric antibodies such as herceptin, an anti-Her-2/neu antibody, in metastatic breast cancer *(259)* is currently the trend. The modifiers discussed here are examples of agents that may prove to be useful in human HCC therapy, but this remains to be seen.

More than half of human HCCs overexpress TGF-α while simultaneously expressing normal or elevated levels of the EGFR, suggesting that an autocrine loop between TGF-α and its receptor may operate in HCC tumor development in the majority of cases. Thus, inhibition of EGFR signaling may be one mechanism to regulate tumor growth. To this end, a humanized chimeric antibody has been developed which appears to bind to the EGFR, inhibit kinase activation, promote receptor internalization, and increase p27KIP1 protein levels resulting in G1 cell cycle arrest. Its administration to patients with various cancers yielded promising results, and further studies are underway. A small molecule inhibitor of EGFR kinase activity has likewise been demonstrated to have some clinical utility and is being investigated *(260)*.

Normal apoptotic and cell cycle control mechanisms seem to be routinely circumvented in human HCCs, notably through mutation and loss of the p53 and Rb genes as well as through alteration of the TGF-β- and Fas-mediated pathways. One promising strategy that may be pertinent to HCC therapy is the use of pharmacological molecules that promote normalized function of mutant p53 proteins through their stabilization resulting in growth arrest *(208)*. Another strategy may be to inhibit the cdks-1 and -2 by 7-hydroxystaurosporine or flavopiridol, both of which are currently under evaluation in humans with various cancers. They may work particularly well in combination with conventional chemotherapeutic drugs *(124)*.

The mechanisms causing hypervascularity and invasion of HCCs may likewise provide good targets for small molecule therapy. Multiple endogenous and synthetic anti-angiogenic molecules exist, and one of the synthetic molecules has received considerable attention and is currently being evaluated in humans with hematological as well as other malignancies *(261)*. This molecule was also shown to significantly inhibit tumor growth in rat models of HCC *(262)*, particularly during early phases of HCC development. ECM remodeling and tumor growth and invasion appear to be modulated by MMPs in human HCC *(263)*. Despite promise

in animal models including those of HCC *(264)*, metalloproteinase inhibitors have not been shown to be effective anti-tumor agents in humans and, in fact, resulted in reduced patient survival in some instances *(265)*.

12. CONCLUSIONS

Due to the rising incidence of HCC combined with the large number of patients who present with advanced disease and the poor response rate of these patients to current treatments, a search for alternative therapies to HCC is underway. Molecular characterization of human HCCs has pointed to numerous aberrant signaling and regulatory pathways, and dissecting these pathways should provide a logical framework for new drug development. To this end, a plethora of molecular agents that target some of them are being clinically evaluated in patients with various tumors. For HCC as well as other tumor types, it may be that a combinatorial approach using new and established agents or multiple new agents, rather than the administration of any single anti-cancer agent, will prove to be most effective because tumor resistance to some small molecule monotherapies has been noted. Melding the knowledge from molecular studies of HCC with the output of promising novel therapies into targeted therapeutic strategies for those with HCC should ultimately have a positive effect on patient care and outcome.

13. REFERENCES

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999;340:745–750.
2. Taylor-Robinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979-94. *Lancet* 1997;350:1142–1143.
3. Hussain SA, Ferry DR, El-Gazzaz G, et al. Hepatocellular carcinoma. Ann Oncol 2001;12:161–172.
4. Di Bisceglie AM, Carithers RL, Jr., Gores GJ. Hepatocellular carcinoma. *Hepatology* 1998;28:1161–1165.
5. Thorgeirsson SS. Mechanism(s) of hepatocarcinogensis: insight from transgenic mouse models. In: *TheLiver Biology and Pathobiology* (Arias IM, ed.), Lippincott Williams & Wilkins, Philadelphia,2001, 1013–1028.
6. Puisieux A, Ozturk M. TP53 and hepatocellular carcinoma. *Pathologie et Biologie* 1997;45:864–870.
7. Salvucci M, Lemoine A, Saffroy R, et al. Microsatellite instability in European hepatocellular carcinoma. *Oncogene* 1999;18:181–187.
8. Buendia MA. Genetics of hepatocellular carcinoma. *Semin Cancer Biol* 2000;10:185–200.
9. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;276:60–66.
10. Kimura M, Ogihara M. Effects of insulin-like growth factor I and II on DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. *Eur J Pharmacol* 1998;354:271–281.
11. Stolz DB, Michalopoulos GK. Comparative effects of hepatocyte growth factor and epidermal growth factor on motility, morphology, mitogenesis, and signal transduction of primary rat hepatocytes. *J Cell Biochem* 1994;55:445–464.

12. Michalopoulos GK, Bowen WC, Zajac VF, et al. Morphogenetic events in mixed cultures of rat hepatocytes and nonparenchymal cells maintained in biological matrices in the presence of hepatocyte growth factor and epidermal growth factor. *Hepatology* 1999;29:90–100.
13. Webster CR, Anwer MS. Phosphoinositide 3-kinase, but not mitogen-activated protein kinase, pathway is involved in hepatocyte growth factor-mediated protection against bile acid-induced apoptosis in cultured rat hepatocytes. *Hepatology* 2001;33:608–615.
14. Wang X, DeFrances MC, Dai Y, et al. A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. *Molecular Cell* 2002;9:411–421.
15. Naldini L, Vigna E, Narsimhan RP, et al. Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. *Oncogene* 1991;6:501–504.
16. Uehara Y, Minowa O, Mori C, et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 1995;373:702–705.
17. Schmidt C, Bladt F, Goedecke S, et al. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995;373:699–702.
18. Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995;376:768–771.
19. Suzuki K, Hayashi N, Yamada Y, et al. Expression of the c-met protooncogene in human hepatocellular carcinoma. *Hepatology* 1994;20:1231–1236.
20. Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor c-met proto-oncogene in hepatocellular carcinoma. *Hepatology* 1997;25:862–866.
21. Tavian D, De Petro G, Benetti A, Portolani N, Giulini SM, Barlati S. u-PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte growth factor mRNA is down-regulated in human hepatocellular carcinoma. *Int J Cancer* 2000;87:644–649.
22. Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res* 1999;59:307–310.
23. Collonge-Rame MA, Bresson-Hadni S, Koch S, et al. Pattern of chromosomal imbalances in non-B virus related hepatocellular carcinoma detected by comparative genomic hybridization. *Cancer Genet Cytogenet* 2001;127:49–52.
24. Rao UN, Gollin SM, Beaves S, Cieply K, Nalesnik M, Michalopoulos GK. Comparative genomic hybridization of hepatocellular carcinoma: correlation with fluorescence *in situ* hybridization in paraffin-embedded tissue. *Molecular Diag* 2001;6:27–37.
25. Kiss A, Wang NJ, Xie JP, Thorgeirsson SS. Analysis of transforming growth factor (TGF)-alpha/epidermal growth factor receptor, hepatocyte growth Factor/c-met,TGF-beta receptor type II, and p53 expression in human hepatocellular carcinomas. *Clin Cancer Res* 1997;3:1059–1066.
26. Bell A, Chen Q, DeFrances MC, Michalopoulos GK, Zarnegar R. The five amino acid-deleted isoform of hepatocyte growth factor promotes carcinogenesis in transgenic mice. *Oncogene* 1999;18:887–895.
27. Brown KD. The epidermal growth factor/transforming growth factor-alpha family and their receptors. *Eur J Gastroenterol Hepatol* 1995;7:914–922.
28. Komuves LG, Feren A, Jones AL, Fodor E. Expression of epidermal growth factor and its receptor in cirrhotic liver disease. *J Histochem Cytochem* 2000;48:821–830.
29. Motoo Y, Sawabu N, Nakanuma Y. Expression of epidermal growth factor and fibroblast growth factor in human hepatocellular carcinoma: an immunohistochemical study. *Liver* 1991;11:272–277.

30. Hisaka T, Yano H, Haramaki M, Utsunomiya I, Kojiro M. Expressions of epidermal growth factor family and its receptor in hepatocellular carcinoma cell lines: relationship to cell proliferation. *Int J Oncol* 1999;14:453–460.
31. Lee GH, Merlino G, Fausto N. Development of liver tumors in transforming growth factor alpha transgenic mice. *Cancer Res* 1992;52:5162–5170.
32. Webber EM, Wu JC, Wang L, Merlino G, Fausto N. Overexpression of transforming growth factor-alpha causes liver enlargement and increased hepatocyte proliferation in transgenic mice. *Am J Pathol* 1994;145:398–408.
33. Hsia CC, Axiotis CA, Di Bisceglie AM, Tabor E. Transforming growth factor-alpha in human hepatocellular carcinoma and coexpression with hepatitis B surface antigen in adjacent liver. *Cancer* 1992;70:1049–1056.
34. Collier JD, Guo K, Gullick WJ, Bassendine MF, Burt AD. Expression of transforming growth factor alpha in human hepatocellular carcinoma. *Liver* 1993;13:151–155.
35. Schaff Z, Hsia CC, Sarosi I, Tabor E. Overexpression of transforming growth factor-alpha in hepatocellular carcinoma and focal nodular hyperplasia from European patients. *Hum Pathol* 1994;25:644–651.
36. Chung YH, Kim JA, Song BC, et al. Expression of transforming growth factor-alpha mRNA in livers of patients with chronic viral hepatitis and hepatocellular carcinoma. *Cancer* 2000;89:977–982.
37. Harada K, Shiota G, Kawasaki H. Transforming growth factor-alpha and epidermal growth factor receptor in chronic liver disease and hepatocellular carcinoma. *Liver* 1999;19:318–325.
38. Ito Y, Takeda T, Sakon M, et al. Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br J Cancer* 2001;84:1377–1383.
39. Morimitsu Y, Hsia CC, Kojiro M, Tabor E. Nodules of less-differentiated tumor within or adjacent to hepatocellular carcinoma: relative expression of transforming growth factor-alpha and its receptor in the different areas of tumor. *Hum Pathol* 1995;26:1126–1132.
40. Hamazaki K, Yunoki Y, Tagashira H, Mimura T, Mori M, Orita K. Epidermal growth factor receptor in human hepatocellular carcinoma. *Cancer Detect Prev* 1997;21:355–360.
41. Baxter RC. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. *Am J Physiol Endocrinol Metab* 2000;278:E967–E976.
42. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Nat Cancer Inst* 2000;92:1472–1489.
43. Giovannone B, Scaldaferri ML, Federici M, et al. Insulin receptor substrate (IRS) transduction system: distinct and overlapping signaling potential. *Diabetes Metab Res Rev* 2000;16:434–441.
44. Jirtle RL. Genomic imprinting and cancer. *Exp Cell Res* 1999;248:18–24.
45. Kalscheuer VM, Mariman EC, Schepens MT, Rehder H, Ropers HH. The insulin-like growth factor type-2 receptor gene is imprinted in the mouse but not in humans. *Nat Genet* 1993;5:74–78.
46. Takeda S, Kondo M, Kumada T, et al. Allelic-expression imbalance of the insulin-like growth factor 2 gene in hepatocellular carcinoma and underlying disease. *Oncogene* 1996;12:1589–1592.
47. Aihara T, Noguchi S, Miyoshi Y, et al. Allelic imbalance of insulin-like growth factor II gene expression in cancerous and precancerous lesions of the liver. *Hepatology* 1998;28:86–89.
48. Sohda T, Yun K, Iwata K, Soejima H, Okumura M. Increased expression of insulin-like growth factor 2 in hepatocellular carcinoma is primarily regulated at the transcriptional level. *Lab Invest* 1996;75:307–311.
49. Ng IO, Lee JM, Srivastava G, Ng M. Expression of insulin-like growth factor II mRNA in hepatocellular carcinoma. *J Gastroenterol Hepatol* 1998;13:152–157.

50. D'Errico A, Grigioni WF, Fiorentino M, et al. Expression of insulin-like growth factor II (IGF-II) in human hepatocellular carcinomas: an immunohistochemical study. Pathol *Int* 1994;44:131–137.
51. Gong Y, Cui L, Minuk GY. The expression of insulin-like growth factor binding proteins in human hepatocellular carcinoma. *Mol Cell Biochem* 2000;207:101–104.
52. Okabe H, Satoh S, Kato T, et al. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res* 2001;61:2129–2137.
53. Hanafusa T, Yumoto Y, Nouso K, et al. Reduced expression of insulin-like growth factor binding protein-3 and its promoter hypermethylation in human hepatocellular carcinoma. *Cancer Lett* 2002;176:149–158.
54. Sasaki Y, Zhang XF, Nishiyama M, Avruch J, Wands JR. Expression and phosphorylation of insulin receptor substrate 1 during rat liver regeneration. *J Biol Chem* 1993;268:3805–3808.
55. Tanaka S, Mohr L, Schmidt EV, Sugimachi K, Wands JR. Biological effects of human insulin receptor substrate-1 overexpression in hepatocytes. *Hepatology* 1997;26:598–604.
56. Nishiyama M, Wands JR. Cloning and increased expression of an insulin receptor substrate-1-like gene in human hepatocellular carcinoma. *Biochem Biophys Res Comm* 1992;183:280–285.
57. Fry MJ. Structure, regulation and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* 1994;1226:237–268.
58. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489–501.
59. Toker A, Newton AC. Cellular signaling: pivoting around PDK-1. *Cell* 2000;103:185–188.
60. West KA, Castillo SS, Dennis PA. Activation of the PI3K/Akt pathway and chemotherapeutic resistance. *Drug Res Updates* 2002;5:234–248.
61. Sun H, Lesche R, Li DM, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. *Proc Nat Acad Sci USA* 1999;96:6199–6204.
62. Skouteris GG, Georgakopoulos E. Hepatocyte growth factor-induced proliferation of primary hepatocytes is mediated by activation of phosphatidylinositol 3-kinase. *Biochem Biophys Res Comm* 1996;218:229–233.
63. Hong F, Nguyen VA, Shen X, Kunos G, Gao B. Rapid activation of protein kinase B/Akt has a key role in antiapoptotic signaling during liver regeneration. *Biochem Biophys Res Comm* 2000;279:974–979.
64. Fruman DA, Mauvais-Jarvis F, Pollard DA, et al. Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. *Nat Genet* 2000;26:379–382.
65. Thorgeirsson SS, Teramoto T, Factor VM. Dysregulation of apoptosis in hepatocellular carcinoma. *Semin Liver Dis* 1998;18:115–122.
66. Yao YJ, Ping XL, Zhang H, et al. PTEN/MMAC1 mutations in hepatocellular carcinomas. *Oncogene* 1999;18:3181–3185.
67. Wan XW, Jiang M, Cao HF, et al. The alteration of PTEN tumor suppressor expression and its association with the histopathological features of human primary hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003;129:100–106.
68. Hu TH, Huang CC, Lin PR, et al. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. *Cancer* 2003;97:1929–1940.
69. Podsypanina K, Ellenson LH, Nemes A, et al. Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Nat Acad Sci USA* 1999;96:1563–1568.
70. Smalley MJ, Dale TC. Wnt signalling in mammalian development and cancer. *Cancer Metastasis Rev* 1999;18:215–230.

71. Monga SP, Pediaditakis P, Mule K, Stolz DB, Michalopoulos GK. Changes in WNT / beta-catenin pathway during regulated growth in rat liver regeneration. *Hepatology* 2001;33:1098–1109.
72. Behrens J. Control of beta-catenin signaling in tumor development. *Ann NY Acad Sci* 2000;910:21–33; discussion 33–35.
73. Monga SP, Mars WM, Pediaditakis P, et al. Hepatocyte growth factor induces Wnt-independent nuclear translocation of beta-catenin after Met-beta-catenin dissociation in hepatocytes. *Cancer Res* 2002;62:2064–2071.
74. He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281:1509–1512.
75. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999;398:422–426.
76. Cui J, Zhou X, Liu Y, Tang Z. Mutation and overexpression of the beta-catenin gene may play an important role in primary hepatocellular carcinoma among Chinese people. *J Cancer Res Clin Oncol* 2001;127:577–581.
77. Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 2000;157:763–770.
78. Prange W, Breuhahn K, Fischer F, et al. Beta-catenin accumulation in the progression of human hepatocarcinogenesis correlates with loss of E-cadherin and accumulation of p53, but not with expression of conventional WNT-1 target genes. *J Pathol* 2003;201:250–259.
79. Ban KC, Singh H, Krishnan R, Seow HF. GSK-3beta phosphorylation and alteration of beta-catenin in hepatocellular carcinoma. *Cancer Lett* 2003;199:201–208.
80. Chen TC, Hsieh LL, Ng KF, Jeng LB, Chen MF. Absence of APC gene mutation in the mutation cluster region in hepatocellular carcinoma. *Cancer Lett* 1998;134:23–28.
81. Piao Z, Kim H, Jeon BK, Lee WJ, Park C. Relationship between loss of heterozygosity of tumor suppressor genes and histologic differentiation in hepatocellular carcinoma. *Cancer* 1997;80:865–872.
82. Su LK, Abdalla EK, Law CH, Kohlmann W, Rashid A, Vauthey JN. Biallelic inactivation of the APC gene is associated with hepatocellular carcinoma in familial adenomatous polyposis coli. *Cancer* 2001;92:332–339.
83. Satoh S, Daigo Y, Furukawa Y, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat Genet* 2000;24:245–250.
84. Laurent-Puig P, Legoix P, Bluteau O, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001;120:1763–1773.
85. Shibata T, Chuma M, Kokubu A, Sakamoto M, Hirohashi S. EBP50, a beta-catenin-associating protein, enhances Wnt signaling and is over-expressed in hepatocellular carcinoma. *Hepatology* 2003;38:178–186.
86. Slagle BL, Zhou YZ, Birchmeier W, Scorsone KA. Deletion of the E-cadherin gene in hepatitis B virus-positive Chinese hepatocellular carcinomas. *Hepatology* 1993;18:757–762.
87. Matsumura T, Makino R, Mitamura K. Frequent down-regulation of E-cadherin by genetic and epigenetic changes in the malignant progression of hepatocellular carcinomas. *Clin Cancer Res* 2001;7:594–599.
88. Endo K, Ueda T, Ueyama J, Ohta T, Terada T. Immunoreactive E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular carcinoma: relationships with tumor grade, clinicopathologic parameters, and patients' survival. *Hum Pathol* 2000;31:558–565.
89. Kanai Y, Ushijima S, Hui AM, et al. The E-cadherin gene is silenced by CpG methylation in human hepatocellular carcinomas. *Int J Cancer* 1997;71:355–359.

90. Jiao W, Miyazaki K, Kitajima Y. Inverse correlation between E-cadherin and Snail expression in hepatocellular carcinoma cell lines in vitro and in vivo. *Br J Cancer* 2002;86:98–101.
91. Li LC, Chui RM, Sasaki M, et al. A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities. *Cancer Res* 2000;60:873–876.92. Cadoret A, Ovejero C, Saadi-Kheddouci S, et al. Hepatomegaly in transgenic mice expressing an oncogenic form of beta-catenin. *Cancer Res* 2001;61:3245–3249.
93. Harada N, Miyoshi H, Murai N, et al. Lack of tumorigenesis in the mouse liver after adenovirus-mediated expression of a dominant stable mutant of beta-catenin. *Cancer Res* 2002;62:1971–1977.
94. Pruitt K, Der CJ. Ras and Rho regulation of the cell cycle and oncogenesis. *Cancer Lett* 2001;171:1–10.
95. Auer KL, Contessa J, Brenz-Verca S, et al. The Ras/Rac1/Cdc42/SEK/JNK/c-Jun cascade is a key pathway by which agonists stimulate DNA synthesis in primary cultures of rat hepatocytes. *Mol Biol Cell* 1998;9:561–573.
96. Cruise JL, Muga SJ, Lee YS, Michalopoulos GK. Regulation of hepatocyte growth: alpha-1 adrenergic receptor and ras p21 changes in liver regeneration. *J Cell Physiol* 1989;140:195–201.
97. Ng YK, Taborn G, Ahmad I, Radosevich J, Bauer K, Iannaccone P. Spatiotemporal changes in Ha-ras p21 expression through the hepatocyte cell cycle during liver regeneration. *Dev Biol* 1992;150:352–362.
98. Ellis CA, Clark G. The importance of being K-Ras. *Cell Signal* 2000;12:425–434.
99. Evans DM, Williams WJ, Kung IT. Angiosarcoma and hepatocellular carcinoma in vinyl chloride workers. *Histopathology* 1983;7:377–388.
100. Weihrauch M, Benicke M, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Frequent k- ras -2 mutations and p16(INK4A)methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. *Br J Cancer* 2001;84:982–989.
101. Tada M, Omata M, Ohto M. Analysis of ras gene mutations in human hepatic malignant tumors by polymerase chain reaction and direct sequencing. *Cancer Res* 1990;50:1121–1124.
102. Leon M, Kew MC. Analysis of ras gene mutations in hepatocellular carcinoma in southern African blacks. *Anticancer Res* 1995;15:859–861.
103. Ogata N, Kamimura T, Asakura H. Point mutation, allelic loss and increased methylation of c-Ha-ras gene in human hepatocellular carcinoma. *Hepatology* 1991;13:31–37.
104. Nonomura A, Ohta G, Hayashi M, et al. Immunohistochemical detection of ras oncogene p21 product in liver cirrhosis and hepatocellular carcinoma. *Am J Gastroenterol* 1987;82:512–518.
105. Jagirdar J, Nonomura A, Patil J, Thor A, Paronetto F. ras oncogene p21 expression in hepatocellular carcinoma. *J Exper Pathol* 1989;4:37–46.
106. Emi M, Fujiwara Y, Ohata H, et al. Allelic loss at chromosome band 8p21.3-p22 is associated with progression of hepatocellular carcinoma. *Genes Chromosomes Cancer* 1993;7:152–157.
107. Qin LX, Tang ZY, Sham JS, et al. The association of chromosome 8p deletion and tumor metastasis in human hepatocellular carcinoma. *Cancer Res* 1999;59:5662–5665.
108. Yuan BZ, Miller MJ, Keck CL, Zimonjic DB, Thorgeirsson SS, Popescu NC. Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. *Cancer Res* 1998;58:2196–2199.
109. Ng IO, Liang ZD, Cao L, Lee TK. DLC-1 is deleted in primary hepatocellular carcinoma and exerts inhibitory effects on the proliferation of hepatoma cell lines with deleted DLC-1. *Cancer Res* 2000;60:6581–6584.
110. Wong CM, Lee JM, Ching YP, Jin DY, Ng IO. Genetic and epigenetic alterations of DLC-1 gene in hepatocellular carcinoma. *Cancer Res* 2003;63:7646–7651.

111. Lin YW, Sheu JC, Liu LY, et al. Loss of heterozygosity at chromosome 13q in hepatocellular carcinoma: identification of three independent regions. *Eur J Cancer* 1999;35:1730–1734.
112. Ching YP, Wong CM, Chan SF, et al. Deleted in liver cancer (DLC) 2 encodes a RhoGAP protein with growth suppressor function and is underexpressed in hepatocellular carcinoma. *J Biol Chem* 2003;278:10,824—10,830.
113. Eisenman RN. Deconstructing myc. *Genes Develop* 2001;15:2023–2030.
114. Pelengaris S, Rudolph B, Littlewood T. Action of Myc in vivo - proliferation and apoptosis. Current Opinion in *Genet Develop* 2000;10:100–105.
115. Chandar N, Lombardi B, Locker J. c-myc gene amplification during hepatocarcinogenesis by a choline-devoid diet. *Proc Nat Acad Sci USA* 1989;86:2703–2707.
116. Pascale RM, De Miglio MR, Muroni MR, et al. c-myc amplification in pre-malignant and malignant lesions induced in rat liver by the resistant hepatocyte model. *Int J Cancer* 1996;68:136–142.
117. Cartier N, Miquerol L, Tulliez M, et al. Diet-dependent carcinogenesis of pancreatic islets and liver in transgenic mice expressing oncogenes under the control of the L-type pyruvate kinase gene promoter. *Oncogene* 1992;7:1413–1422.
118. Fourel G, Trepo C, Bougueleret L, et al. Frequent activation of N-myc genes by hepadnavirus insertion in woodchuck liver tumours. *Nature* 1990;347:294–298.
119. Hsu T, Moroy T, Etiemble J, et al. Activation of c-myc by woodchuck hepatitis virus insertion in hepatocellular carcinoma. *Cell* 1988;55:627–635.
120. Abou-Elella A, Gramlich T, Fritsch C, Gansler T. c-myc amplification in hepatocellular carcinoma predicts unfavorable prognosis. *Mod Pathol* 1996;9:95–98.
121. Kawate S, Fukusato T, Ohwada S, Watanuki A, Morishita Y. Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic features, proliferative activity and p53 overexpression. *Oncology* 1999;57:157–163.
122. Gan FY, Gesell MS, Alousi M, Luk GD. Analysis of ODC and c-myc gene expression in hepatocellular carcinoma by in situ hybridization and immunohistochemistry. *J Histochem Cytochem* 1993;41:1185–1196.
123. Yuen MF, Wu PC, Lai VC, Lau JY, Lai CL. Expression of c-Myc, c-Fos, and c-jun in hepatocellular carcinoma. *Cancer* 2001;91:106–112.
124. Sampath D, Plunkett W. Design of new anticancer therapies targeting cell cycle checkpoint pathways. *Curr Opin Oncol* 2001;13:484–490.
125. Daujat S, Neel H, Piette J. MDM2: life without p53. *Trends Genet* 2001;17:459–464.
126. Kishimoto Y, Shiota G, Kamisaki Y, et al. Loss of the tumor suppressor p53 gene at the liver cirrhosis stage in Japanese patients with hepatocellular carcinoma. *Oncology* 1997;54:304–310.
127. Tanaka S, Toh Y, Adachi E, Matsumata T, Mori R, Sugimachi K. Tumor progression in hepatocellular carcinoma may be mediated by p53 mutation. *Cancer Res* 1993;53:2884–2887.
128. Park NH, Chung YH, Youn KH, et al. Close correlation of p53 mutation to microvascular invasion in hepatocellular carcinoma. *J Clin Gastroenterol* 2001;33:397–401.
129. Yano M, Asahara T, Dohi K, Mizuno T, Iwamoto KS, Seyama T. Close correlation between a p53 or hMSH2 gene mutation in the tumor and survival of hepatocellular carcinoma patients. *Int J Oncol* 1999;14:447–451.
130. Ng IO, Fan ST. Is the p53 gene mutation of prognostic value in hepatocellular carcinoma? [letter; comment.]. *Arch Surg* 2000;135:1476.
131. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991;350:429–431.
132. Ozturk M. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. *Lancet* 1991;338:1356–1359.

133. Feitelson MA, Zhu M, Duan LX, London WT. Hepatitis B x antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene* 1993;8:1109–1117.
134. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Nat Acad Sci USA* 1994;91:2230–2234.
135. Becker SA, Lee TH, Butel JS, Slagle BL. Hepatitis B virus X protein interferes with cellular DNA repair. *J Virol* 1998;72:266–272.
136. Pogribny IP, James SJ. Reduction of p53 gene expression in human primary hepatocellular carcinoma is associated with promoter region methylation without coding region mutation. *Cancer Lett* 2002;176:169–174.
137. Bourdon JC, D'Errico A, Paterlini P, Grigioni W, May E, Debuire B. p53 protein accumulation in European hepatocellular carcinoma is not always dependent on p53 gene mutation. *Gastroenterology* 1995;108:1176–1182.
138. Qiu SJ, Ye SL, Wu ZQ, Tang ZY, Liu YK. The expression of the mdm2 gene may be related to the aberration of the p53 gene in human hepatocellular carcinoma. *J Cancer Res Clin Oncol* 1998;124:253–258.
139. Endo K, Ueda T, Ohta T, Terada T. Protein expression of MDM2 and its clinicopathological relationships in human hepatocellular carcinoma. *Liver* 2000;20:209–215.
140. Nevins JR. The Rb/E2F pathway and cancer. *Hum Mol Genet* 2001;10:699–703.
141. Zhang X, Xu HJ, Murakami Y, et al. Deletions of chromosome 13q, mutations in Retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. *Cancer Res* 1994;54:4177–4182.
142. Ashida K, Kishimoto Y, Nakamoto K, et al. Loss of heterozygosity of the retinoblastoma gene in liver cirrhosis accompanying hepatocellular carcinoma. *J Cancer Res Clin Oncol* 1997;123:489–495.
143. Roncalli M, Borzio M, Bianchi P, Laghi L. Comprehensive allelotype study of hepatocellular carcinoma. *Hepatology* 2000;32:876.
144. Hada H, Koide N, Morita T, et al. Promoter-independent loss of mRNA and protein of the Rb gene in a human hepatocellular carcinoma. *Hepatogastroenterology* 1996;43:1185–1189.
145. Edamoto Y, Hara A, Biernat W, et al. Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. *Int J Cancer* 2003;106:334–341.
146. Higashitsuji H, Itoh K, Nagao T, et al. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. *Nat Med* 2000;6:96–99.
147. Fu XY, Wang HY, Tan L, Liu SQ, Cao HF, Wu MC. Overexpression of p28/gankyrin in human hepatocellular carcinoma and its clinical significance. *W J Gastroenterol* 2002;8:638–643.
148. Hui AM, Li X, Makuuchi M, Takayama T, Kubota K. Over-expression and lack of retinoblastoma protein are associated with tumor progression and metastasis in hepatocellular carcinoma. *Int J Cancer* 1999;84:604–608.
149. Naka T, Toyota N, Kaneko T, Kaibara N. Protein expression of p53, p21WAF1, and Rb as prognostic indicators in patients with surgically treated hepatocellular carcinoma. *Anticancer Res* 1998;18:555–564.
150. Zimonjic DB, Keck CL, Thorgeirsson SS, Popescu NC. Novel recurrent genetic imbalances in human hepatocellular carcinoma cell lines identified by comparative genomic hybridization. *Hepatology* 1999;29:1208–1214.
151. Hussussian CJ, Struewing JP, Goldstein AM, et al. Germline p16 mutations in familial melanoma. *Nat Genet* 1994;8:15–21.

152. Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA. Role of the INK4a locus in tumor suppression and cell mortality. *Cell* 1996;85:27–37.
153. Bates S, Phillips AC, Clark PA, et al. p14ARF links the tumour suppressors RB and p53. *Nature* 1998;395:124–125.
154. Liew CT, Li HM, Lo KW, et al. High frequency of p16INK4A gene alterations in hepatocellular carcinoma. *Oncogene* 1999;18:789–795.
155. Jin M, Piao Z, Kim NG, et al. p16 is a major inactivation target in hepatocellular carcinoma. *Cancer* 2000;89:60–68.
156. Piao Z, Park C, Lee JS, Yang CH, Choi KY, Kim H. Homozygous deletions of the CDKN2 gene and loss of heterozygosity of 9p in primary hepatocellular carcinoma. *Cancer Lett* 1998;122:201–207.
157. Hui AM, Sakamoto M, Kanai Y, et al. Inactivation of p16INK4 in hepatocellular carcinoma. *Hepatology* 1996;24:575–579.
158. Hui AM, Shi YZ, Li X, Takayama T, Makuuchi M. Loss of p16(INK4) protein, alone and together with loss of retinoblastoma protein, correlate with hepatocellular carcinoma progression. *Cancer Lett* 2000;154:93–99.
159. Chaubert P, Gayer R, Zimmermann A, et al. Germ-line mutations of the p16INK4(MTS1) gene occur in a subset of patients with hepatocellular carcinoma. *Hepatology* 1997;25:1376–1381.
160. Lee TK, Man K, Ling MT, et al. Over-expression of Id-1 induces cell proliferation in hepatocellular carcinoma through inactivation of p16INK4a/RB pathway. *Carcinogenesis* 2003;24:1729–1736.
161. Dotto GP. p21(WAF1/Cip1): more than a break to the cell cycle? *Biochim et Biophys Acta* 2000;1471:M43–M56.
162. Hui AM, Kanai Y, Sakamoto M, Tsuda H, Hirohashi S. Reduced p21(WAF1/CIP1) expression and p53 mutation in hepatocellular carcinomas. *Hepatology* 1997;25:575–579.
163. Furutani M, Arii S, Tanaka H, et al. Decreased expression and rare somatic mutation of the CIP1/WAF1 gene in human hepatocellular carcinoma. *Cancer Lett* 1997;111:191–197.
164. Kobayashi S, Matsushita K, Saigo K, et al. P21WAF1/CIP1 messenger RNA expression in hepatitis B, C virus-infected human hepatocellular carcinoma tissues. *Cancer* 2001;91:2096–2103.
165. Qin LF, Ng IO. Expression of p27(KIP1) and p21(WAF1/CIP1) in primary hepatocellular carcinoma: clinicopathologic correlation and survival analysis. *Hum Pathol* 2001;32:778–784.
166. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Develop* 1999;13:1501–1512.
167. Philipp-Staheli J, Payne SR, Kemp CJ. p27(Kip1): regulation and function of a haploinsufficient tumor suppressor and its misregulation in cancer. *Exper Cell Res* 2001;264:148–168.
168. Tannapfel A, Grund D, Katalinic A, et al. Decreased expression of p27 protein is associated with advanced tumor stage in hepatocellular carcinoma. *Int J Cancer* 2000;89:350–355.
169. Ito Y, Matsuura N, Sakon M, et al. Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. *Hepatology* 1999;30:90–99.
170. Fiorentino M, Altimari A, D'Errico A, et al. Acquired expression of p27 is a favorable prognostic indicator in patients with hepatocellular carcinoma. *Clin Cancer Res* 2000;6:3966–3972.
171. Catzavelos C, Bhattacharya N, Ung YC, et al. Decreased levels of the cell-cycle inhibitor p27Kip1 protein: prognostic implications in primary breast cancer. *Nat Med* 1997;3:227–230.

172. Kusano N, Shiraishi K, Kubo K, Oga A, Okita K, Sasaki K. Genetic aberrations detected by comparative genomic hybridization in hepatocellular carcinomas: their relationship to clinicopathological features. *Hepatology* 1999;29:1858–1862.
173. Zhang YJ, Jiang W, Chen CJ, et al. Amplification and overexpression of cyclin D1 in human hepatocellular carcinoma. *Biochem Biophys Res Commun* 1993;196:1010–1016.
174. Nishida N, Fukuda Y, Komeda T, et al. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994;54:3107–3110.
175. Yamashita T, Kaneko S, Hashimoto S, et al. Serial analysis of gene expression in chronic hepatitis C and hepatocellular carcinoma. *Biochem Biophys Res Commun* 2001;282:647–654.
176. Deane NG, Parker MA, Aramandla R, et al. Hepatocellular carcinoma results from chronic cyclin D1 overexpression in transgenic mice. *Cancer Res* 2001;61:5389–5395.
177. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature* 2001;411:342–348.
178. Gupta S. Molecular steps of death receptor and mitochondrial pathways of apoptosis. *Life Sci* 2001;69:2957–2964.
179. Ni R, Tomita Y, Matsuda K, et al. Fas-mediated apoptosis in primary cultured mouse hepatocytes. *Exper Cell Res* 1994;215:332–337.
180. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al. Lethal effect of the anti-Fas antibody in mice. *Nature* 1993;364:806–809.
181. Oberhammer FA, Pavelka M, Sharma S, et al. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. *Proc Natl Acad Sci USA* 1992;89:5408–5412.
182. Bissell DM, Roulot D, George J. Transforming growth factor beta and the liver. *Hepatology* 2001;34:859–867.
183. Zhang X, Wang T, Batist G, Tsao MS. Transforming growth factor beta 1 promotes spontaneous transformation of cultured rat liver epithelial cells. *Cancer Res* 1994;54:6122–6128.
184. Factor VM, Kao CY, Santoni-Rugiu E, Woitach JT, Jensen MR, Thorgeirsson SS. Constitutive expression of mature transforming growth factor beta 1 in the liver accelerates hepatocarcinogenesis in transgenic mice. *Cancer Res* 1997;57:2089–2095.
185. Ito N, Kawata S, Tamura S, et al. Elevated levels of transforming growth factor beta messenger RNA and its polypeptide in human hepatocellular carcinoma. *Cancer Res* 1991;51:4080–4083.
186. Shirai Y, Kawata S, Tamura S, et al. Plasma transforming growth factor-beta 1 in patients with hepatocellular carcinoma. Comparison with chronic liver diseases. *Cancer* 1994;73:2275–2279.
187. Sue SR, Chari RS, Kong FM, et al. Transforming growth factor-beta receptors and mannose 6-phosphate/insulin-like growth factor-II receptor expression in human hepatocellular carcinoma. *Ann Surg* 1995;222:171–178.
188. Furuta K, Misao S, Takahashi K, et al. Gene mutation of transforming growth factor beta1 type II receptor in hepatocellular carcinoma. *Int J Cancer* 1999;81:851–853.
189. Kawate S, Takenoshita S, Ohwada S, et al. Mutation analysis of transforming growth factor beta type II receptor, Smad2, and Smad4 in hepatocellular carcinoma. *Int J Oncology* 1999;14:127–131.
190. Yakicier MC, Irmak MB, Romano A, Kew M, Ozturk M. Smad2 and Smad4 gene mutations in hepatocellular carcinoma. *Oncogene* 1999;18:4879–4883.
191. Dennis PA, Rifkin DB. Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. *Proc Natl Acad Sci USA* 1991;88:580–584.

192. Dahms NM, Lobel P, Kornfeld S. Mannose 6-phosphate receptors and lysosomal enzyme targeting. *J Biol Chem* 1989;264:12,115—12,118.
193. De Souza AT, Hankins GR, Washington MK, Fine RL, Orton TC, Jirtle RL. Frequent loss of heterozygosity on 6q at the mannose 6-phosphate/insulin-like growth factor II receptor locus in human hepatocellular tumors. *Oncogene* 1995;10:1725–1729.
194. Piao Z, Choi Y, Park C, Lee WJ, Park JH, Kim H. Deletion of the M6P/IGF2r gene in primary hepatocellular carcinoma. *Cancer Lett* 1997;120:39–43.
195. Yamada T, De Souza AT, Finkelstein S, Jirtle RL. Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. *Proc Natl Acad Sci USA* 1997;94:10,351—10,355.
196. Wada I, Kanada H, Nomura K, Kato Y, Machinami R, Kitagawa T. Failure to detect genetic alteration of the mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) gene in hepatocellular carcinomas in Japan. *Hepatology* 1999;29:1718–1721.
197. De Souza AT, Hankins GR, Washington MK, Orton TC, Jirtle RL. M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. *Nat Genet* 1995;11:447–449.
198. Kaplowitz N. Cell death at the millennium. Implications for liver diseases. *Clin Liver Dis* 2000;4:1–23, v.
199. Natoli G, Ianni A, Costanzo A, et al. Resistance to Fas-mediated apoptosis in human hepatoma cells. *Oncogene* 1995;11:1157–1164.
200. Ito Y, Monden M, Takeda T, et al. The status of Fas and Fas ligand expression can predict recurrence of hepatocellular carcinoma. *Br J Cancer* 2000;82:1211–1217.
201. Kubo K, Matsuzaki Y, Okazaki M, Kato A, Kobayashi N, Okita K. The Fas system is not significantly involved in apoptosis in human hepatocellular carcinoma. *Liver* 1998;18:117–123.
202. Lee SH, Shin MS, Lee HS, et al. Expression of Fas and Fas-related molecules in human hepatocellular carcinoma. *Hum Pathol* 2001;32:250–256.
203. Roskams T, Libbrecht L, Van Damme B, Desmet V. Fas and Fas ligand: strong co-expression in human hepatocytes surrounding hepatocellular carcinoma; can cancer induce suicide in peritumoural cells? *J Pathol* 2000;191:150–153.
204. Takehara T, Hayashi N. Fas and fas ligand in human hepatocellular carcinoma. *J Gastroenterol* 2001;36:727–728.
205. Ito T, Shiraki K, Sugimoto K, et al. Survivin promotes cell proliferation in human hepatocellular carcinoma. *Hepatology* 2000;31:1080–1085.
206. Chen GG, Lai PB, Chan PK, et al. Decreased expression of Bid in human hepatocellular carcinoma is related to hepatitis B virus X protein. *Eur J Cancer* 2001;37:1695–1702.
207. Fujikawa K, Shiraki K, Sugimoto K, et al. Reduced expression of ICE/caspase1 and CPP32/caspase3 in human hepatocellular carcinoma. *Anticancer Res* 2000;20:1927–1932.
208. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* 2002;108:153–164.
209. Rojkind MaG, Patricia. The extracellular matrix of the liver. In: Arias IM, ed. *The Liver Biology and Pathobiology*. New York: Raven Press, 1994:843–868.
210. Martinez-Hernandez A, Delgado FM, Amenta PS. The extracellular matrix in hepatic regeneration. Localization of collagen types I, III, IV, laminin, and fibronectin. *Lab Invest* 1991;64:157–166.
211. Ruoslahti E. Integrins. *J Clin Invest* 1991;87:1–5.
212. Roskelley CD, Srebrow A, Bissell MJ. A hierarchy of ECM-mediated signalling regulates tissue-specific gene expression. *Curr Opin Cell Biol* 1995;7:736–747.
213. Frisch SM, Screaton RA. Anoikis mechanisms. *Curr Opin Cell Biol* 2001;13:555–562.

214. Rojkind MaG, Patricia. Pathophysiology fo liver fibrosis. In: Arias IM, ed. *The Liver Biology and Pathobiology*. Philadelphia: Lippincott Williams & Wilkins, 2001:721–738.
215. Le Bail B, Faouzi S, Boussarie L, Balabaud C, Bioulac-Sage P, Rosenbaum J. Extracellular matrix composition and integrin expression in early hepatocarcinogenesis in human cirrhotic liver. *J Pathol* 1997;181:330–337.
216. Torimura T, Ueno T, Kin M, et al. Coordinated expression of integrin alpha6beta1 and laminin in hepatocellular carcinoma. *Hum Pathol* 1997;28:1131–1138.
217. Murphy G, Gavrilovic J. Proteolysis and cell migration: creating a path? *Curr Opin Cell Biol* 1999;11:614–621.
218. Giannelli G, Bergamini C, Fransvea E, Marinosci F, Quaranta V, Antonaci S. Human hepatocellular carcinoma (HCC) cells require both alpha3beta1 integrin and matrix metalloproteinases activity for migration and invasion. *Lab Invest* 2001;81:613–627.
219. Nakashima O, Kojiro M. Recurrence of hepatocellular carcinoma: multicentric occurrence or intrahepatic metastasis? A viewpoint in terms of pathology. *J Hepatobiliary Pancreat Surg* 2001;8:404–409.
220. Kirimlioglu H, Dvorchick I, Ruppert K, et al. Hepatocellular carcinomas in native livers from patients treated with orthotopic liver transplantation: biologic and therapeutic implications. *Hepatology* 2001;34:502–510.
221. Rudolph KL, Trautwein C, Kubicka S, et al. Differential regulation of extracellular matrix synthesis during liver regeneration after partial hepatectomy in rats. *Hepatology* 1999;30:1159–1166.
222. Kim TH, Mars WM, Stolz DB, Michalopoulos GK. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* 2000;31:75–82.
223. Andreasen PA, Kjoller L, Christensen L, Duffy MJ. The urokinase-type plasminogen activator system in cancer metastasis: a review. *Int J Cancer* 1997;72:1–22.
224. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993;143:949–958.
225. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem* 1997;378:151–160.
226. McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 2001;13:534–540.
227. Morita Y, Hayashi Y, Wang Y, et al. Expression of urokinase-type plasminogen activator receptor in hepatocellular carcinoma. *Hepatology* 1997;25:856–861.
228. Ashida K, Nakatsukasa H, Higashi T, et al. Cellular distribution of 92-kd type IV collagenase/gelatinase B in human hepatocellular carcinoma. *Am J Pathol* 1996;149:1803–1811.
229. Arii S, Mise M, Harada T, et al. Overexpression of matrix metalloproteinase 9 gene in hepatocellular carcinoma with invasive potential. *Hepatology* 1996;24:316–322.
230. Sakamoto Y, Mafune K, Mori M, et al. Overexpression of MMP-9 correlates with growth of small hepatocellular carcinoma. *Int J Oncol* 2000;17:237–743.
231. Giannelli G, Bergamini C, Marinosci F, et al. Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. *Int J Cancer* 2002;97:425–431.
232. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–257.
233. Sugimachi K, Tanaka S, Terashi T, Taguchi K, Rikimaru T. The mechanisms of angiogenesis in hepatocellular carcinoma: angiogenic switch during tumor progression. *Surgery* 2002;131:S135—S141.
234. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;407:242–248.
235. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocrine Rev* 1997;18:4–25.

236. Ng IO, Poon RT, Lee JM, Fan ST, Ng M, Tso WK. Microvessel density, vascular endothelial growth factor and its receptors Flt-1 and Flk-1/KDR in hepatocellular carcinoma. *Am J Clin Pathol* 2001;116:838–845.
237. Miura H, Miyazaki T, Kuroda M, et al. Increased expression of vascular endothelial growth factor in human hepatocellular carcinoma. *J Hepatol* 1997;27:854–861.
238. Chow NH, Hsu PI, Lin XZ, et al. Expression of vascular endothelial growth factor in normal liver and hepatocellular carcinoma: an immunohistochemical study. *Hum Pathol* 1997;28:698–703.
239. Moon WS, Rhyu KH, Kang MJ, et al. Overexpression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? *Mod Pathol* 2003;16:552–557.
240. Shimamura T, Saito S, Morita K, et al. Detection of vascular endothelial growth factor and its receptor expression in human hepatocellular carcinoma biopsy specimens. *J Gastroenterol Hepatol* 2000;15:640–646.
241. Loughna S, Sato TN. Angiopoietin and Tie signaling pathways in vascular development. *Matrix Biol* 2001;20:319–325.
242. Tanaka S, Mori M, Sakamoto Y, Makuuchi M, Sugimachi K, Wands JR. Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. *J Clin Invest* 1999;103:341–345.
243. Tanaka S, Sugimachi K, Yamashita Yi Y, et al. Tie2 vascular endothelial receptor expression and function in hepatocellular carcinoma. *Hepatology* 2002;35:861–867.
244. Soff GA. Angiostatin and angiostatin-related proteins. *Cancer Metastasis Rev* 2000;19:97–107.
245. Dong Z, Kumar R, Yang X, Fidler IJ. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* 1997;88:801–810.
246. Gorrin Rivas MJ, Arii S, Furutani M, et al. Expression of human macrophage metalloelastase gene in hepatocellular carcinoma: correlation with angiostatin generation and its clinical significance. *Hepatology* 1998;28:986–993.
247. Suehiro T, Terashi T, Shiotani S, Soejima Y, Sugimachi K. Liver transplantation for hepatocellular carcinoma. *Surgery* 2002;131:S190—S194.
248. Bergsland EK, Venook AP. Hepatocellular carcinoma. *Curr Opin Oncol* 2000;12:357–361.
249. Okuda K. Hepatocellular carcinoma. *J Hepatol* 2000;32:225–237.
250. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997;336:1855–1859.
251. Omata M, Shiratori Y. Long-term effects of interferon therapy on histology and development of hepatocellular carcinoma in hepatitis C. *J Gastroenterol Hepatol* 2000;15:E134–E140.
252. Scott LJ, Perry CM. Interferon-alpha-2b plus ribavirin: a review of its use in the management of chronic hepatitis C. *Drugs* 2002;62:507–556.
253. Muto Y, Moriwaki H, Ninomiya M, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med* 1996;334:1561–1567.
254. Muto Y, Moriwaki H, Saito A. Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. *N Engl J Med* 1999;340:1046–1047.
255. Okuno M, Sano T, Matsushima-Nishiwaki R, et al. Apoptosis induction by acyclic retinoid: a molecular basis of 'clonal deletion' therapy for hepatocellular carcinoma. *Japan J Clin Oncol* 2001;31:359–362.
256. Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002;20:1692–1703.

257. Scollay R. Gene therapy: a brief overview of the past, present, and future. *Ann NY Acad Sci* 2001;953:26–30.
258. Qian C, Drozdzik M, Caselmann WH, Prieto J. The potential of gene therapy in the treatment of hepatocellular carcinoma. *J Hepatol* 2000;32:344–351.
259. Baselga J, Albanell J. Mechanism of action of anti-HER2 monoclonal antibodies. *Ann Oncol* 2001;12:S35–S41.
260. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001;7:2958–2970.
261. Liekens S, De Clercq E, Neyts J. Angiogenesis: regulators and clinical applications. *Biochem Pharmacol* 2001;61:253–270.
262. Kin M, Torimura T, Ueno T, et al. Angiogenesis inhibitor TNP-470 suppresses the progression of experimentally-induced hepatocellular carcinoma in rats. *Int J Oncol* 2000;16:375–382.
263. Ikebe T, Yamamoto T, Kubo S, et al. Suppressive effect of the angiogenesis inhibitor TNP-470 on the development of carcinogen-induced hepatic nodules in rats. *Japan J Cancer Res* 1998;89:143–149.
264. Bu W, Tang ZY, Sun FX, et al. Effects of matrix metalloproteinase inhibitor BB-94 on liver cancer growth and metastasis in a patient-like orthotopic model LCI-D20. *Hepatogastroenterology* 1998;45:1056–1061.
265. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 2002;295:2387–2392.

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