Molecular Mechanisms of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) typically has poor prognosis, because it is often diagnosed at an advanced stage. Heterogeneous phenotypic and genetic traits of affected individuals and a wide range of risk factors have classified it a complex disease. HCC is not amenable to standard chemotherapy and is resistant to radiotherapy. In most cases, surgical resection and liver transplantation remain the only curative treatment options. Therefore, development of novel, effective therapies is of prime importance. Extensive research over the past decade has identified a number of molecular biomarkers as well as cellular networks and signaling pathways affected in liver cancer. Recent studies using a combination of “omics” technologies, microRNA studies, combinatorial chemistry, and bioinformatics are providing new insights into the gene expression and protein profiles during various stages of the disease. In this review, we discuss the contribution of these newer approaches toward an understanding of molecular mechanisms of HCC and for the development of novel cancer therapeutics.

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Abbreviations: 2D-GE, two-dimensional gel electrophoresis; AFB1, aflatoxin B1; CDK, cyclin-dependent kinase; cDNA, complementary DNA; CSC, cancer stem cell; ERK, extracellular-regulated kinase; EHR, early intrahepatic recurrence; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDB, high-dimensional biology; HPLC, high-performance liquid chromatography; HSP, heat shock protein; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MAPK, mitogen-activated protein kinase; miRNA, microRNA; mRNA, messenger RNA; MS, mass spectrometry; MudPIT, multidimensional protein identification technology; PTEN, phosphatase and tensin homolog; RISC, RNA-induced silencing complex; SELDI, surface-enhanced laser desorption/ionization; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.

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A variety of risk factors have been associated with HCC. Such factors include exposure to hepatitis viruses,1-3 vinyl chloride,4 tobacco,5 foodstuffs contaminated with aflatoxin B1 (AFB1),6 heavy alcohol intake,7 nonalcoholic fatty liver disease,8 diabetes,9,10 obesity,10 diet,11 coffee,12 oral contraceptives,13 and hemochromatosis.14 In general, these factors vary according to the geographical region, adding complexity to the extrapolation of data obtained from one region and applying it to others. For example, chronic hepatitis B virus (HBV) infection is prevalent in many Asian countries and Africa, whereas hepatitis C virus (HCV) is dominant in Japan and the United States.1

The pathophysiology of HCC is not understood clearly, but underlying liver dysfunction, especially cirrhosis, is a predisposing condition. Hepatocarcinogenesis is a complex process associated with accumulation of genetic and epigenetic changes that occur during initiation, promotion, and progression of the disease. Cellular events are often accompanied by increased expression of several factors that influence the survival of cancerous cells by suppressing apoptosis and regulating cell cycle. Activation of oncogenes and the role of tumor suppressor genes such as retinoblastoma and p53 genes have also been well documented. The growing incidence of HCC has generated intense research to understand the physiological, cellular, and molecular mechanisms of the disease with the hope of developing new treatment strategies.

Cellular Signaling Pathways Involved in Hepatocarcinogenesis

Extensive epidemiological studies over the years have identified major risk factors of HCC and many advances
have been made to understand the pathogenesis of HCC. However, little is known about molecular mechanisms that lead to carcinogenesis. Abrupt changes that occur in liver tissues due either to viral infection or exposure to hepatotoxic agents cause significant changes in the cellular signaling pathways and alter gene expression resulting in tumor formation (Fig. 1). Signal transduction pathways implicated in HCC (Table 1) are briefly reviewed here. These pathways are being studied extensively to identify potential biomarkers and molecular targets (Table 2).

**Wnt/β-Catenin Pathway.** The Wnt signaling pathway, originally identified in *Drosophila melanogaster*, is highly conserved in evolutionary pathways involved in homeostasis, cell proliferation, differentiation, motility, and apoptosis. It is deregulated in a number of cancers, including HCC. In most cases, either the inactivation of the tumor suppressor gene adenomatous polyposis coli or mutation of the proto-oncogene β-catenin and the activation of Wnt signaling was observed. This pathway is involved in HCC arising from HBV/HCV infections and alcoholic liver cirrhosis. Up-regulation of frizzled-7 and dephosphorylation of β-catenin is frequently observed in HCC. Therefore, targeted inactivation of Wnt pathway is a potential therapeutic target for cancer.

Mutations in β-catenin do promote the activation of Wnt/β-catenin signaling, though they prevent its phosphorylation and subsequent degradation. Mutant protein normally accumulates in the nucleus, and its presence correlates with low incidence of HCC. Furthermore, mutations in β-catenin arise in HCC patients with increased exposure to HCV infection and aflatoxin. In addition to these mutations in β-catenin, mutations in Axin 1 and Axin 2, negative regulators of Wnt pathway,

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**Table 1. Cellular Signaling Pathways Known to Be Affected in HCC Caused by Various Risk Factors**

| Risk Factor       | Pathway Affected          | Reference |
|-------------------|---------------------------|-----------|
| Aflatoxin         | Wnt/β-catenin             | 28        |
|                   | p53                       | 35, 37    |
| Alcohol           | Wnt/β-catenin             | 24        |
| HBV               | Wnt/β-catenin             | 24        |
|                   | p53                       | 37        |
|                   | p53                       | 43, 44    |
|                   | MAP kinase                | 49        |
|                   | Cytokine signaling        | 68        |
| HCV               | Wnt/β-catenin             | 24        |
|                   | MAP kinase                | 49        |
| Hemochromatosis   | p53                       | 38        |
| Chemical carcinogen| Ras                      | 52-58     |

**Table 2. Potential Biomarkers Associated with Various Risk Factors Causing HCC and Their Association with Cellular Signaling Pathways**

| Risk Factor       | Biomarker                          | Reference |
|-------------------|------------------------------------|-----------|
| Aflatoxin         | Mutations in β-catenin             | 27        |
|                   | Mutations in codon 249 of p53      | 35        |
|                   | Dephosphorylation of β-catenin     | 24        |
|                   | Up-regulation of frizzled-7        | 24        |
|                   | Down-regulation of pRb             | 43, 44    |
|                   | Down-regulation of p16             | 44        |
| HBV               | Dephosphorylation of β-catenin     | 24        |
|                   | Mutations in β-catenin             | 27        |
|                   | Up-regulation of frizzled-7        | 24        |
| HCV               | Dephosphorylation of β-catenin     | 24        |
|                   | Mutations in β-catenin             | 27        |
|                   | Up-regulation of frizzled-7        | 24        |
| Hemochromatosis   | Mutations in codons 249 and 250 of p53 | 38 |
| Chemical carcinogen| Point mutation in codon 12 of N-ras | 53        |
|                   | Point mutation in codon 61 of K-ras | 53        |
|                   | Point mutation in codon 13 of H-ras | 54, 55    |
|                   | Point mutation in codon 64 of K-ras | 56        |
were also observed in HCC.\textsuperscript{23,24} A liver-specific disruption of adenomatous polyposis coli gene in mice was shown to result in the activation of Wnt pathway.\textsuperscript{25} Thus, Wnt/β-catenin pathway is an important signaling pathway in HCC.

**p53 Pathway.** In about half of all human tumors, the tumor suppressor TP53 gene is inactivated by a single point mutation.\textsuperscript{26} In the remaining cancers, p53 is expressed at normal levels but the p53 signaling that leads to cell cycle arrest and subsequent apoptosis is defective. Loss of p53 function also sensitizes cells to checkpoint signals.\textsuperscript{27} In general, cellular levels of p53 are low; however, in response to intracellular and extracellular stress signals, p53 expression is up-regulated. DNA-damaging agents, such as ultraviolet or gamma irradiation and chemotherapeutic drugs, also activate p53 by covalent modification, including phosphorylation of the transactivation domain and acetylation and phosphorylation of basic allosteric control region by ataxia telangiectasia mutated and related kinases.\textsuperscript{28}

Several studies have reported that p53 mutations and inactivation play a critical role in HCC. For instance, in a clinical study of 16 Chinese patients with HCC, 8 had a point mutation at the third base position of codon 249. Moreover, the G→T transversion in seven HCC DNA samples and the G→C transversion in the other HCC were consistent with mutations caused by AFB\textsubscript{1} in mutagenesis experiments, and no mutations were found in exons 5, 6, or 8 or in the remainder of exon 7.\textsuperscript{29} This mutational hotspot in codon 259 was also found in several human cancer cell lines.\textsuperscript{30} In a case-control study, serum hepatitis B surface antigen and liver AFB\textsubscript{1}-DNA adducts were found to be significantly elevated in HCC samples compared with controls.\textsuperscript{31} Furthermore, all mutations in the codon 249 occurred in hepatitis B surface antigen–seropositive carriers, and mutations in p53 DNA and protein correlated to tumor stage, suggesting that they are late events. Thus, detection of mutant p53 in plasma serves as a potential biomarker for AFB\textsubscript{1} exposure and presence of HCC.

The effect of oxidative stress in liver carcinogenesis, most notably in hemochromatosis and Wilson disease, was shown to be associated with p53 mutations.\textsuperscript{32} Under such conditions, oxidative stress results in the development of cirrhosis with a 200-fold risk for HCC. Mutations that commonly alter p53 function include G:C to T:A transversions at codon 249 as well as to C:T and C:G to T:A transversions at codon 250. Furthermore, an elevated level of inducible nitric oxide synthase expression was also reported in this study.

**pRB Pathway.** The tumor suppressor retinoblastoma protein pRb1 is a major cellular barrier to cancer development.\textsuperscript{33} It controls cell cycle progression via repression of the E2F transcription factor family of proteins. The activity of cyclin-dependent kinases (CDKs) correlates with the onset of pRB phosphorylation and G1/S cell cycle transition.\textsuperscript{34} Up to 16 possible CDK phosphorylation sites exist on pRB, and multiple CDKs can phosphorylate pRB with some site specificity.\textsuperscript{35} Mutational inactivation of both \textit{Rb1} alleles is the primary molecular alteration that causes the pediatric cancer retinoblastoma. Hereditary retinoblastoma is produced by germline transmission of one mutually inactive \textit{Rb1} allele and loss of the remaining wild-type allele in somatic retinal cells. Hence hereditary retinoblastoma typically has an earlier onset and a greater number of tumor foci than sporadic retinoblastoma where both \textit{Rb1} alleles must be inactivated in somatic retinal cells.\textsuperscript{33,35} Furthermore, a strong correlation between loss of pRB and lack of functional p53 was observed in early studies on human tumors.\textsuperscript{36} Taken together with the fact that several DNA tumor viruses, such as human papilloma viruses, encode proteins that inactivate both pRB and p53, it has been suggested that loss of pRB results in p53-dependent apoptosis.

The CDK inhibitors p16\textsuperscript{INK4A}, p21\textsuperscript{WAF1/CIP1}, and p27\textsuperscript{Kip1} are independently affected and a change in the expression of one or more of these inhibitors contributes to carcinogenesis in nearly 90% of HCC cases.\textsuperscript{37,38} p16\textsuperscript{INK4A} is predominantly inactivated during the early stages of hepatocarcinogenesis as well as in disease progression. Reduced p21\textsuperscript{WAF1/CIP1} expression, which is associated mainly with p53 gene mutation in HCCs, also contributes to hepatocarcinogenesis. Several studies have demonstrated that the pRB pathway is severely disrupted in HCC patients. When pRB expression was analyzed in 25 patients with HBV-induced HCC using histochemical staining, it was found that pRB expression was altered in eight patients. Among these, pRB was not detectable in five cases, whereas in three others <1% of nuclei were stained for pRB.\textsuperscript{37} Another study examined the expression of pRB, cyclin D1, and p16 in 47 HCC specimens and found that 38 of them had been inactivated in either pRB or p16 expression, whereas cyclin D1 was overexpressed in only five samples.\textsuperscript{38} This disruption in the pRB pathway in HCC was similar to that observed in various cancers, demonstrating that pRB is a critical player in carcinogenesis.

**Mitogen-Activated Protein Kinase Pathway.** The intracellular mitogen-activated protein kinase (MAPK) family has five MAPK subgroups. These include the extracellular signal-regulated kinase protein homologs 1 and 2 (ERK1/2), big MAPK-1 (BMK-1/ERK5), c-Jun N-terminal kinase homologs 1, 2, and 3 (JNK1/2/3), stress-
activated protein kinase 2 (SAPK-2) homologs α, β, and δ (p38α/β/δ), and ERK6, also known as p38γ.39 The activity of these kinases is dependent upon dual phosphorylation of T and Y residues located in their activation loop. MAPKs were implicated in diverse cellular processes such as cell survival, differentiation, adhesion, and proliferation.40,41

Proteins of HBV, HCV, and hepatitis E virus modulate MAPK signaling by targeting multiple steps along the signaling pathway.42 For instance, HCV E2 protein activates the MAPK pathway in human hepatoma Huh-7 cells and promotes cell proliferation.43 In human HCC, the expression levels of Spred protein (Sprouty-related protein with Ena/vasodilator-stimulated phosphoprotein homology-1 domain), an inhibitor of the Ras/Raf-1/ERK pathway, are deregulated.44 Forced expression of Spred caused inhibition of ERK activation both in vivo and in vitro, resulting in reduced proliferation of cancer cells and low secretion of matrix metalloproteinases 2 and 9. This finding shows direct correlation of MAPK-ERK pathway activation and HCC, suggesting that Spred could serve as a therapeutic target for human HCC.

Ras Pathway. Human ras proteins H-Ras, N-Ras, K-ras4A, and K-ras4B are small GTP-binding proteins that function as molecular switches to influence cell growth, differentiation and apoptosis.45 Single point mutations in codon 13 of H-ras, codon 12 of N-ras, and codon 61 of K-ras were originally observed in HCC caused by various chemicals such as N-nitrosomorpholine, bleomycin, 1-nitropyrene, and methyl (acetoxyethyl) nitrosamine.46-52 Ras interacts with a downstream serine/threonine kinase Raf-1 leading to its activation and downstream signaling, which includes activation of MAPK kinases MEK1 and MEK2, to regulate proliferation of cancer cells and low secretion of matrix metalloproteinases 2 and 9. This finding shows direct correlation of MAPK-ERK pathway activation and HCC, suggesting that Spred could serve as a therapeutic target for human HCC.

JAK/STAT Pathway. Signal transducers and activators of transcription (STATs) comprise a family of transcription factors that are activated by a variety of cytokines, hormones, and growth factors.60 Their activation occurs through tyrosine phosphorylation by Janus kinases (JAKs). Activated STATs stimulate the transcription of suppressors of cytokine signaling (SOCS) genes. SOCS proteins, in turn, bind phosphorylated JAKs and their receptors to inhibit this pathway, thereby preventing overactivation of cytokine-stimulated cells.61 Thus, SOCS are part of the negative feedback loop in the JAK/STAT circuitry. Two other families of STAT inhibitors that are described in the literature include the protein inhibitors of activated STATs and the SH2-containing proteins.62 JAK stimulation of STATs activates cell proliferation, migration, differentiation, and apoptosis, and deregulation of inhibitors leads to human diseases, including cancer.63 Inactivation of SOCS-1 and SSI-1, a JAK-binding protein, in HCC have been reported,64,65 as has the ubiquitous activation of the JAK/STAT pathway.64

Stress Response Signaling. Heat shock proteins (HSPs) are critical players in cellular stress response. Under stress conditions, they undergo phosphorylation and/or dephosphorylation. In a recent study conducted with 48 clinical specimens, HCC progression was found to be associated with the decrease in serine phosphorylation of HSP27.65 In another study with 146 clinical specimens, several members of the HSP family were found to be associated with the occurrence of HCC,66 suggesting that HSPs are key players in HCC progression.

Epidermal Growth Factor Receptor and Transforming Growth Factor-β Pathways. The molecular dynamics of HCC can also be influenced by proteins and cellular factors of other signaling pathways. For example, vascular endothelial growth factor and fibroblast growth factor play important roles in HCC development.57,68 It was reported recently that inflammation is inherently associated with cancer and a number of cytokines are involved in promoting HCC development and progression, especially during infection with hepatitis viruses.59 In particular, Th2 cytokines are induced and Th1 cytokines decreased in metastases. Therefore, modulating the expression of cytokines and the use of inhibitors of inflammatory cytokines might be critical in alleviating HCC progression. In a recent study, it was shown that the use of inhibitors of epidermal growth factor receptor and transforming growth factor β prevented the development of HCC in rat liver, demonstrating the harmful nature of these growth factors if they exist in excessive amounts.70,71

Others. Lack of apoptotic cell death is a hallmark of cancer. By suppressing the expression of an antiapoptotic myeloid cell leukemia-1 protein using RNA interference, it was demonstrated that induction of apoptosis can be achieved in HCC cancer cells in vitro.72 Additional pathways of physiological processes such as alcohol metabolism, cellular transport, and ubiquitins may play a role in regulating hepatocarcinogenesis.
Table 3. Technologies Used for Molecular Profiling of Biomolecules

| OMICS | TECHNOLOGIES |
|-------|--------------|
| Genomics | Comparative genomic hybridization, Suppression subtractive hybridization, Polymerase chain reaction-based microsatellite instability assay |
| Transcriptomics | cDNA microarray (also termed Gene Chip), Two-dimensional gel electrophoresis |
| Proteomics | Mass spectrometry, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Surface-enhanced laser desorption/ionization mass spectrometry, Stable isotope labeling with amino acids in cell culture, Isotope-coated affinity tag, Isobaric tags for relative and absolute quantification, High-performance liquid chromatography, Strong anion exchange chromatography, Immobilized metal-ion affinity chromatography, Metal oxide affinity chromatography, Protein microarray (also termed Protein Chip), Antibody microarray, Reverse-phase microarray, Multidimensional protein identification technology |
| Metabolomics | Nuclear magnetic resonance spectroscopy, Direct infusion electrospray-MS, Gas chromatography, Liquid chromatography, Fourier transform infra-red spectroscopy, Raman spectroscopy |

The “Omics” Revolution

The success of genome sequencing project in the late 1990s have ushered in a new era of “omics” with the aim of understanding the complexity of a cell, tissue, and an organism in its entirety. New disciplines such as genomics and proteomics as well as a broader research area of systems biology are taking shape in an explosive manner. The principle tenet of systems biology is to capture the data derived from the omics technologies and integrate them with theoretical models to predict the behavior of an organism. More recently, high-dimensional biology (HDB) was introduced for simultaneous study of thousands of variables using high-throughput omics methods from the same sample data set. Such studies aim to understand the physiology or mechanisms of a disease. In contrast to systems biology, HDB involves a relatively smaller sample size and does not require a hypothesis. Both systems biology and HDB are complementary to each other and the knowledge obtained from them is expected to result in the development of novel diagnostic, prognostic, and therapeutic tools. In recent years, studies of these global-scale methods (Table 3) were instrumental in understanding the molecular mechanisms of HCC. They helped us to identify biomarkers, signaling pathways, cellular networks, and potential therapeutic targets.

Molecular Profiling and Biomarker Identification

Molecular profiling of genes, proteins and other molecules provides powerful tools to gain insight into the molecular mechanisms underlying carcinogenesis. Such profiling allows us to understand the molecular anatomy of normal cells and that of tumor cells. Knowledge obtained from such studies could be translated to develop new diagnostic, prognostic, and therapeutic targets for clinical intervention. Most importantly, systems biology and HDB provide the opportunity to study the complex relationship and coordination between genomes, proteomes, and metabolomes rather than single individual factors. Many studies have focused on the changes in global expression profiles for a large number of cellular genes and proteins in hepatocarcinogenesis. Specific gene-expression signatures obtained from these studies may help to accurately predict the risk for developing HCC. In addition, they may also assist in identifying potential biomarkers and therapeutic targets for elucidating the molecular mechanisms associated with HCC.

Genomics: Chromosomal Instabilities and Epigenetic Modifications. Genetic and epigenetic events that lead to hepatocarcinogenesis are relatively poorly understood. To develop effective therapies and personalized medicine for heterogenous and complex diseases such as liver cancer, a complete understanding of the genotype-phenotype relationship is essential. This can be achieved by elucidating the role of environmental factors and human biology in health and disease. HCC displays gross genomic alterations, including chromosomal instability, CpG methylation, DNA rearrangements associated with HBV integration, DNA hypomethylation, and, to a lesser degree, microsatellite instability. Aberrant patterns of chromosomal changes and other types of biological information such as genomic locus, gene function, and sequence may be used to help classify the complex traits of liver cancer. Notably, the altered transcriptome profiles in HCC could be correlated to several chromosome regions with amplification or loss of heterozygosity (LOH). The next stage of gene expression analysis will require systematic integration of expression profiles with locus information, which is effective in detecting structural genomic abnormalities, such as chromosomal gains and deletions. Array-based comparative genomic hybridization analysis and allelic dosage analysis using genotyping arrays are useful for determining genetic factors involved in HCC. A recent study using integrative analysis of array-based comparative genomic hybridization and expression...
profiling of 20 HCC cell lines and primary tumors reported overexpression of paternally expressed gene 10 (PEG10), a target of c-MYC, located in the chromosome region 7q21 that correlated with the progression of HCC.81

Specific areas of the genome appear unstable in HCC, with the same regions undergoing either deletion or increased gene dosage in all HCC.82 Gains of 1q21–23 and 8q22–24 were identified as genomic events associated with the early development of HCC in a genome-wide study of chromosomal aberrations of 158 HBV-associated HCC cases using comparative genomic hybridization.83 Based on patterns of significant chromosomal aberrations, three HCC subgroups organized in an evolutionary tree were recently identified. These groups reflect the degree of tumor progression, including a number of chromosomal aberrations, tumor stage, tumor size, and disease outcome. Furthermore, the gain of 3q22–24 was identified as one of the late genomic events associated with tumor recurrence and poor overall patient survival.83 Various studies have reported chromosomal instability at chromosomal regions, 1p, 4q, 5q, 6q, 8p, 10q, 11p, 16p, 16q, 17p, and 22q (reviewed by Herath et al.79). Frequent promoter hypermethylation and subsequent loss of protein expression has also been demonstrated in HCC at tumor suppressor gene, p16, p14, p15, SOCS1, RIZ1, E-cadherin, and 14-3-3 sigma. An interesting observation emerging from these studies is the presence of a methylator phenotype in hepatocarcinogenesis.79 Promoter methylation also appears to be an early event, suggesting that this may precede cirrhosis. However, these genes have been studied in isolation and global studies of methylator phenotype are required to assess the significance of epigenetic silencing in hepatocarcinogenesis. DNA hypomethylation, CpG island hypermethylation, and histone modifications represent epigenetic markers of malignant transformation.84

Based on these data, there are obvious fundamental differences in the mechanisms of hepatic carcinogenesis, with at least two distinct mechanisms of malignant transformation in the liver, related to chromosome instability and CpG methylation. The reason for these differences and the relative importance of these mechanisms are unclear but likely relate to the pathogenesis of HCC. Defining these broad mechanisms is a necessary prerequisite for determining the timing of events in malignant transformation of the liver and investigating the role of known risk factors for HCC.

Transcriptomics: Gene Expression Profiling. Accumulation of mutations and alterations in expressed genes often results in carcinogenesis. HCC development and progression caused by genetic changes resulting in altered expression of thousands of cancer-related genes can be measured by global genetic analysis. Gene expression profiling of HCC has been employed to elucidate hepatocarcinogenesis and to identify molecular mechanisms underlying complex clinical features. Identification of phenotype-associated gene profiling will have a significant impact on the diagnosis and management of HCC.85 Common technologies used to study genomics include complementary DNA (cDNA) microarrays to analyze global gene expression, single nucleotide polymorphism genotyping to identify mutations that alter the gene expression and aberrant protein functions, identification of regions of chromosomal instability, and DNA–protein interactions. Several studies have applied these technologies to HCC and have identified candidate genes useful as biomarkers in cancer staging and prediction of recurrence and prognosis, as well as for the development of treatment strategies. Some of these target molecules have also been used to develop new serum diagnostic markers and therapeutic targets against HCC.

A comprehensive characterization of gene expression profiles of HBV-induced HCC was performed recently using a large set of 5′-read expressed sequence tag clusters from HCC and noncancerous liver samples, and by comparing them with a cDNA microarray containing 12,393 genes/expressed sequence tag.78 Integrated data from these analyses identified 2,253 genes/expressed sequence tag as candidates with differential expression. Many genes involved in cell cycle regulation such as cyclins, cyclin-dependent kinases, and cell cycle–negative regulators were deregulated in most patients with HCC, together with molecules of the Wnt/β-catenin pathway and enzymes for DNA replication enzymes and metabolism.78 In this study, in silico resampling analysis of DNA microarray data that reproduced the entire geographic distribution pattern of HBV and HCV in six representative geographic regions was performed. One hundred genes among each virtual cohort were then compared. Many human leukocyte antigen family genes were common among all cohorts, suggesting that this gene family represents the pathway most responsible for early intrahepatic recurrence (EHR) of HCC worldwide.86

A recent investigation utilized the messenger RNA (mRNA) differential display method to examine three paired tumor and nontumor tissues that had chromosomally integrated HBV DNA through chronic infection.87 This study identified 29 known and four novel genes (HA61T2, PT18, HG63T1, and HG57T1) that were differentially overexpressed as well as 27 known and five novel genes (DNT10, PT8, PT19, ENT25, and HA6T4) that were underexpressed in those tumor tissues.87 Overexpression of HG57T1 and low expression of
DNT10 correlated with low serum alpha-feto protein levels, suggesting that these novel genes could be good candidates for biomarkers of HBV-positive HCC.

In another study, gene expression patterns and global genomic alterations in HCC, hepatoblastomas, adjacent nontumor tissues, and tissues derived from healthy livers and hepatic resections were analyzed. Several genes including Glypican 3, spondin-2, PEG10, EDIL3, and Osteopontin were found to be overexpressed in HCC, whereas IGF2, fibronectin, DLK1, transforming growth factor β1, MALAT1, and MIG6 were overexpressed in hepatoblastomas. A similar study designed to assess the transcriptional characteristics of HCC by integrating gene expression data obtained with rat fetal hepatoblasts and adult hepatocytes with HCC from human and mouse models revealed that diseased individuals who shared the gene expression pattern with fetal hepatoblasts had poor prognosis models. Furthermore, analyses of gene networks showed that activation of AP-1 might have a key role in tumor development.

In a study using 11,065 expressed sequence tag clusters from HCC and noncancerous liver samples, genes involved in cell cycle regulation such as cyclins, cyclin-dependent kinases, and cell cycle–negative regulators were found to be deregulated in most specimens. A differential gene expression analysis of various cell lines and human HCC tissues revealed a significant difference in expression levels of 14 genes (FTL, K-ALPHA1, LDHA, RPL4, ENO1, ANXA2, RPL9, RPL10, RPL13A, GNB2L1, AMBP, GC, A1BG, and SERPINC1) between HBV- and HCV-associated HCC. Another study using suppression subtractive hybridization coupled with cDNA microarray analysis revealed that the up-regulated genes in HCC were involved in processes such as transcription, protein biosynthesis, metabolism of lipids and proteins, cell proliferation, and signal transduction, whereas down-regulated genes included liver-synthesized proteins such as fibrinogen and enzymes such as ADH1C, ALDH6A1, ALDODB, Arginase, and CES1. In another study, 13 out of 22 genes differentially expressed in Japanese patients were found to encode either transcriptional factors or tissue-specific expression proteins related to cell differentiation or development. Distribution pattern of HBV and HCV in different geographic regions was recently tested using in silico resampling analysis of DNA microarray data to identify genes associated with early intrahepatic recurrence (EHR) of HCC within 1 year of surgery in six geographic virtual cohorts, each consisting of 1,000 virtual samples. By comparing 100 of the most commonly expressed genes among each virtual cohort, the investigators found that many human leukocyte antigen family genes were present in all six cohorts, suggesting that this gene family represents the pathway most responsible for early intrahepatic recurrence of HCC worldwide.

A molecular analysis of damaged liver tissues infected with HCV from 40 patients using a cDNA microarray identified 230 differentially expressed genes in the multicentric HCC group when compared with the multicentric recurrence group. A study with Affymetrix GeneChip expression arrays to determine differential patterns of gene expression for primary liver carcinoma, metastatic liver carcinoma, and normal liver tissue found that, in comparison with genes of normal liver tissue, 842 genes were overexpressed, and 393 genes were underexpressed in HCC. All these genes were players in diverse cellular processes. Another study analyzed transcriptional profiles of 55 candidate genes using quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) in 17 dysplastic nodules and 20 early HCC specimens from HCV cirrhotic patients undergoing resection/transplantation and compared them with 10 nontumoral cirrhotic tissues and 10 normal liver tissues. Results from this study showed that 12 genes encoding for TERT, GPC3, gankyrin, survivin, TOP2A, LYVE1, E-cadherin, IGFBP3, PDGFRα, transforming growth factor A, cyclin D1, and HGF were differentially expressed in early HCCs when compared with dysplastic nodules. Logistic regression analysis identified the expression of three of these genes (GPC3, LYVE1, and survivin) to be highly significant, suggesting that gene transcriptional profiles of a three-gene set allow a reliable diagnosis for early HCC.

In a large-scale study with 58,251 cDNA clones of full-length cDNA libraries of HBV and HCV-infected HCC and their surrounding nontumor tissues, it was reported that 180 genes were up-regulated and 279 genes were down-regulated between HCC tissue and its adjacent nontumor tissue. The candidate genes encoded for liver-specific metabolism enzymes, secretory functional proteins, proteases and their inhibitors, protein chaperon, cell cycle components, apoptosis-related proteins, transcriptional factors, and DNA binding proteins.

HCC patients with vascular invasion and cirrhosis have a high rate (78% to 83%) of developing recurrent disease within 6 to 35 months after resection. In comparison, most of the HCC patients (80% to 100%) without vascular invasion and cirrhosis remain disease-free. However, the risk of recurrent disease for HCC patients with either vascular invasion or cirrhosis could not be accurately ascertained. Using Affymetrix human HG-U133A and HG-U133B oligonucleotide probe arrays with a pool of cDNA from 23 HCC patients, a 57-gene signature that could predict recurrent disease with 84% accuracy was recently identified.
Activation of Akt-mTORC1 signaling pathway in pre-malignant and HCC lesions was recently examined by assessing the expression of pS6, an Akt effector, and phosphatase and tensin homolog (PTEN), an Akt suppressor, from 52 patients with cirrhosis, with and without HCC.97 Immunohistochemical pS6 staining was shown to be greater in cirrhotic tissue from patients with HCC than in cirrhosis patients without HCC, and PTEN staining in tumor was absent in 24% cases.97 In this report, the relevance of AKT and ERK1/2 in HCC was analyzed in a series of 208 patients treated either by surgical resection or liver transplantation. Activation of ERK1/2 correlated statistically with the presence of HCV infection, and pERK1/2 and pAKT expression showed a significant correlation with a decreased overall survival, suggesting that HCV infection activates the ERK pathway and might contribute to HCC carcinogenesis.98

Gene expression studies were performed in the woodchuck animal model for hepatitis virus-induced HCC using a human oligonucleotide microarray.99 The analyses of gene expression was conducted by combining (1) supervised significant analysis of microarray, (2) prediction analysis of microarray, and (3) unsupervised hierarchical cluster methodologies statistically determined 211 up-regulated and 78 down-regulated genes between liver cancer and noncancer liver tissues. These genes were involved in transcription, RNA splicing, translation, cell cycle, metabolism, protein folding and degradation, apoptosis, immune response, and metal binding. Genes involved in signaling pathways such as Ras/MAPK (MAPKAP1), Src-dependent pathways, and the hedgehog signaling pathway were also differentially expressed in this animal model, while those of the Wnt signaling pathway were down-regulated. In addition to these genes, aberrant expression of p53 and genes coding for DNA replication enzymes was also reported.78 Genome-scale chromosomal copy number alteration profiles and mutations in p53 and β-catenin genes among 87 HCC tumors showed that HCCs with heterogeneous genetic backgrounds harbor distinct genetic alterations, demonstrating the diversity of HCC.100

In contrast to these studies, one study investigated deregulation of housekeeping genes during eight different stages of HCV-induced HCC using microarrays.101 This study revealed differential expression of most housekeeping genes, including glyceraldehyde-3-phosphate dehydrogenase and β-actin. This finding underscores the importance for careful selection of control genes for quantitative PCR, and use of these common genes for normalization may lead to misinterpretation of the results. In another investigation, the gene expression profiles of metastatic disease and EHR—two representative modes of recurrence of HCC attributable to metastasis—were analyzed using DNA microarray analysis. Forty-six signature genes were analyzed for EHR in 35 HCCs that included cell adhesion–related genes (ITGA6, SPP1, DNMBP, CD44, and POSTN) and 10 immune response–related genes that all showed higher expression in HCC with EHR than in HCC without EHR. These results suggest that alteration of the cell adhesion system plays a central role in EHR and that reduction of the immune response is a specific step in early intrahepatic recurrence.102 Collectively, these genomic studies have provided enormous amounts of information on both up- and down-regulation of cellular genes, suggesting that diverse cellular processes are affected by HCC disease states.

**Protein Expression Profiling and Functional Analysis Using Proteomics.** The term proteome is used to refer to the entire set of proteins encoded by the genome of an organism,103 and proteomics is the study of the expression, structure, and function of proteins. Traditionally, the study of protein profiles was performed using two-dimensional gel electrophoresis (2D-GE),104 a process in which proteins are separated in the first dimension by their molecular weight and in the second dimension by their isoelectric point. The proteins identified as unique following comparison between tumor and nontumor samples are excised and studied further using mass spectrometry (MS) approaches.105 2D-GE is a simple and powerful method to visualize thousands of proteins and detect their alterations and posttranslational modifications; however, intergel variation, labor intensiveness, and high cost are major drawbacks. A significant advance in a polyacrylamide gel electrophoresis–based approach has been the fluorescent two-dimensional differential in-gel electrophoresis.106 In this method, different samples are prelabeled with fluorescent cyanine dyes (Cy2, Cy3, or Cy5) that are matched to the mass and charge of proteins and coseparated on the same gel to overcome intergel variations.107 Two-dimensional differential in-gel electrophoresis can yield greater accuracy for quantification than silver staining due to better sensitivity and dynamic range of fluorescent dyes.108 New techniques are being developed to enhance the sensitivity and capacity to handle large-scale proteomic studies. Such methods include matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS,109 surface-enhanced laser desorption/ionization (SELDI),110 stable isotope labeling with amino acids in cell culture, isotope-coated affinity tag, and isobaric tags for relative and absolute quantification. Although these technologies offer good reliability, sensitivity, and reproducibility, they are expensive and are restrictive to subcellular studies. Several chromatography-based methods are also used in proteomics research. They
include high-performance liquid chromatography (HPLC), strong anion exchange chromatography, immobilized metal-ion affinity chromatography, and metal oxide affinity chromatography.111 Protein microarrays112 and antibody-based proteomics113 are versatile methods to analyze protein-protein interactions as well as functional and biochemical activities of proteins on a global scale. Protein arrays can also be applied to study protein-lipid, protein-drug, protein-nucleic acid, and antigen-antibody interactions.114 These technologies allow large-scale and high-throughput studies, but they are of limited accuracy. Apart from SELDI, three other types of protein microarrays that are currently in use are functional protein array, antibody microarray, and reverse-phase microarray.112-116 It is also possible to study protein complexes using MS,117 HPLC/MS technologies have high flexibility, specificity, speed, and reproducibility. However, it is not possible to detect posttranslational modifications or protein-protein interactions with these techniques. Multidimensional protein identification technology (MudPIT)118,119 is a novel technology used to study whole proteomes, organelles, protein complexes, and posttranslational modifications. Complex protein/peptide mixtures can be analyzed by coupling MudPIT MS/MS with biphasic or triphasic HPLC. The major advantage of MudPIT over 2D-GE is its speed.119 The major drawback of MudPIT MS/MS is its inability to detect low-abundance proteins and putative interactions between various proteins. However, with advances in tissue procurement such as laser capture microdissection to enrich tumor cells from tissues, each of these technologies constitutes an important tool for biomarker discovery.

Serum profiling is useful for the early detection and prediction of HCC in patients with cirrhosis and chronic viral infections. A comparative analysis of protein profiles between HCC and adjacent nontumor tissues using 2D-GE and MS identified 47 protein spots corresponding to 23 distinct genes.120 In this study, a positive correlation between transcript and protein level variations was observed for only seven of them, including four endoplasmic reticulum-resident proteins: calreticulin, PDIA3, PDI, and GRP78. All four endoplasmic reticulum proteins were proteolytically cleaved and the fragments were detected in culture supernatant of the hepatoma cell line PLC-PRF5, and calreticulin and PDIA3 cleavage products were detectable in sera of patients with HCC. These findings suggest that cleavage products of endoplasmic reticulum proteins form a new class of biomarker for HCC.

A study of differentially expressed proteins in samples from 12 HBV-associated HCC patients identified 61 spots that were significantly up-regulated and 158 that were down-regulated in HCC. HSP70 and HSP90 family members were up-regulated in HCC samples, whereas metabolism-associated, mitochondrial, and peroxisomal proteins were decreased. Four metabolic enzymes involved in the methylation cycle were also down-regulated in HCC tissues, indicating the occurrence of S-adenosylmethionine deficiency in HCC.111 In another study, peptide fingerprint mapping and SELDI MS separated by 2D-GE were used to identify 53 proteins whose expression was altered in hepatic tumor tissues.121 Among these proteins, three potential diagnostic markers were selected based on a significant change in their expression levels. These include two down-regulated proteins, ferritin light subunit and adenyylate kinase 3 alpha-like 1, and biliverdin reductase B, which was up-regulated.

An investigation aimed at identifying novel diagnostic markers of HCC for early detection of HCC employed SELDI-TOF/MS with serum samples obtained from 153 HCV patients with or without HCC. Use of these diagnostic markers predicted the diagnosis of HCC in six of these seven patients before HCC was clinically apparent without any false positives.122 Protein profile analysis of serum obtained from five HCC patients and five control patients using 2D-GE identified 14 differentially expressed proteins between HCC patients and the control group on the 2D-GE gel. Further analysis of the proteome in human serum using nano-HPLC electrospray ionization tandem MS revealed that only six proteins—complement component 9, ceruloplasmin, annexin VI isoform 1, and three serum amyloids (A4, A2, and A1 isoform 2)—were present in HCC patients. These six proteins might serve as biomarkers for HCC.123 A comparative study of protein profiles between tumorous and nontumorous specimens from HCV patients using 2D-GE revealed 11 spots that were down-regulated. Eight of these 11 spots were subsequently identified as liver type aldolase, tropomyosin beta-chain, ketohexokinase enoyl-CoA hydratase, albumin, smoothelin, ferritin light chain, and arginase 1 using MALDI-TOF. These proteins were suggested to be useful biomarkers for carcinogenesis of HCV-related HCC.124

A number of studies have examined protein profiles in cell lines during hepatitis virus infection. One such study identified cytokeratins (8, 18, and 19), vimentin, HSP90, coflin, and low molecular weight phosphotyrosine protein phosphatase as cellular proteins that interact with HCV core protein using 2D-GE and MALDI-TOF.125 Up-regulation of proteins involved in diverse cellular processes such as cell signaling, protein transport and vesicle formation in HCV-infected HuH7 cells was reported in a study using stable isotope labeling with amino acids in cell culture, polyacrylamide gel electrophoresis, and MS.126
Protein profiling conducted with HCC cell lines also demonstrated alterations in proteins involved in oxidative stress, cytoskeleton, calcium homeostasis, metabolic enzymes, and HSP27.\textsuperscript{127-132}

Taken together, a large number of proteins have been identified as potential markers in HCC specimens by studying their expression profiles using various proteomic approaches. Further studies are needed to determine their diagnostic value.

**Metabolic Processes Affected in HCC.** Metabolic profiling has been used to catalog the functions of metabolites in a specific organ under a given set of conditions, such as cancer. Metabolomics is a discipline that aims to quantify global composition of metabolites and identify specific phenotypes of the tissue, organ, or organism.\textsuperscript{133,134} Because metabolic pathways are downstream of gene expression and protein synthesis, they might reflect the biological activity of a cell at functional levels more accurately.\textsuperscript{134} A number of methods have been described to study metabolites. They include nuclear magnetic resonance spectroscopy,\textsuperscript{135} direct infusion electrospray MS,\textsuperscript{136} gas and liquid chromatography, Fourier transform infra-red spectroscopy,\textsuperscript{137} and Raman spectroscopy.\textsuperscript{138}

When HBV- and HCV-related HCC tissues were compared using proteomics, it was found that enoyl-CoA reductase was reduced in HBV and increased in HCV-associated HCC.\textsuperscript{139} Overexpression of stathmin 1 and proliferating cell nuclear antigen occurred only in HBV, whereas hepatic aldolase B was replaced by nonhepatic isoform A in HCV-infected tissues.\textsuperscript{124} Up-regulation of apolipoprotein E, a protein that alters β-catenin distribution, was also reported for HCV-induced HCC.\textsuperscript{140} Similarly, fructose-bisphosphate aldolase B was down-regulated,\textsuperscript{141} and proteins involved in glucose metabolism and osmoregulation were also differentially expressed in HBV-associated HCC.\textsuperscript{142} Down-regulation of various metabolic enzymes and cathepsin A was also reported. A recent study showed that the ubiquitin-conjugating enzyme E2C (Ube2c) was overexpressed in human HCC at significantly higher levels than in the corresponding non-cancerous tissues. Patients with high Ube2c expression also showed significantly lower disease-free survival rates than those with low Ube2c expression.\textsuperscript{143} Thus, Ube2c is a potential prognostic biomarker for HCC.

**Role of Stem/Progenitor Cells in HCC.** Over the years, it has been well established that both hepatocytes and cholangiocytes are capable of repopulating liver tissue following injury.\textsuperscript{144} Therefore, the concept of stem/progenitor cell existence in the liver did not gain much recognition until the past decade. Furthermore, growing evidence also demonstrated that the capacity to sustain tumor formation and growth resides in a small proportion of cancer stem cells (CSCs).\textsuperscript{145,146} Subsequent identification of CSCs in a number of tissues including brain,\textsuperscript{147-149} prostate,\textsuperscript{150} breast,\textsuperscript{151} myeloid,\textsuperscript{152} gastric,\textsuperscript{153} colon,\textsuperscript{154,155} and lung,\textsuperscript{156} has reinforced the notion that stem cells might also exist in the liver. Several laboratories have successfully isolated stem/progenitor cells from human, primate, hamster, and rodent livers, both from normal and cancerous tissues.\textsuperscript{157-161}

In the early studies, embryonic stem cells from murine embryos were shown to differentiate into functional hepatocytes \textit{in vitro}.\textsuperscript{162,163} It was later shown that murine as well as human bone marrow–derived mesenchymal stem cells could differentiate into hepatocytes both \textit{in vitro} and \textit{in vivo}.\textsuperscript{164,165} Studies of bone marrow transplant recipients have shown that these cells could home to liver and differentiate into normal hepatocytes.\textsuperscript{166,167} One of the most common liver stem cells is the oval cell.\textsuperscript{168-171} Oval cells express markers common to hepatocytes and cholangiocytes, suggesting that they are bipotential. In fact, they differentiate into hepatocytes and cholangiocytes \textit{in vivo} under the appropriate culture conditions.\textsuperscript{172} In diseases such as alcoholic liver disease and HCV infection, oval cell numbers increase and correlate with the severity of the disease.\textsuperscript{173} Several groups have isolated liver progenitor cell lines using oval cells from choline-deficient diet-fed rats,\textsuperscript{172} c-met transgenic mice,\textsuperscript{173} p53 null mice,\textsuperscript{174} and murine embryonic liver cells.\textsuperscript{175} Successful isolation of oval cells and establishment of liver progenitor cell lines from human liver tumors\textsuperscript{176} and isolation of CSCs from human cell lines have been reported.\textsuperscript{177} The presence of CSCs and successful isolation of oval cells from cancerous tissue suggests that stem/progenitor cells play a key role in tumor formation. Recently, a novel cell type, the liver-derived progenitor cell, was also discovered and was isolated from healthy, uninjured rat livers.\textsuperscript{178} Further studies with these progenitor cells may provide insight to understand the molecular events that regulate cellular differentiation of the liver and those that lead to tumor progression.

**Involvement of MicroRNAs in Hepatocarcinogenesis.** Identification of small, noncoding RNAs in the early 1990s has led to the development of a new research area of RNomics.\textsuperscript{179} Several different classes of noncoding RNAs have been discovered in mammalian cells. These include small interfering RNAs,\textsuperscript{180} small nucleolar RNAs,\textsuperscript{181} and microRNAs (miRNAs).\textsuperscript{182} miRNAs are initially produced by RNA polymerase II as primary precursor transcripts that form a stem–loop structure and undergo processing by a protein complex containing the RNase III enzyme Drosha and the double-stranded RNA-binding protein Pasha in the nucleus (Fig. 2). These pro-
cessed precursors (pre-miRNAs) are then exported into the cytoplasm by exportin-5, where they undergo further cleavage to form mature miRNAs. miRNAs are then loaded onto an RISC complex and are directed to 3′ untranslated region of target mRNAs. miRNA binds to its complementary sequence in 3′ untranslated region and causes either translational inhibition or mRNA degradation.

Recent studies have demonstrated that alterations in miRNA genes lead to tumor formation, and several miRNAs that regulate either the tumor suppression or promote tumor formation have been identified. For example, down-regulation of miR-15 and miR-16 results in overexpression of bcl2, cdk6, and cdc27, whereas overexpression of miR-21 causes suppression of PTEN and TP53. Several miRNAs that regulate the tumor suppressor p53 and p53-responsive genes have also been identified. Among these, miR-34 regulates p53 function in cell cycle arrest, cellular senescence, and apoptosis. It has been demonstrated that miR-127 is strongly silenced and/or down-regulated in cancer cells. This silencing was found to be mediated by hypermethylation of the putative miRNA promoter region and could be reversed only by the combination of a DNA demethylating agent (5-aza-2′-deoxycytidine) and a histone deacetylase inhibitor (4-phenylbutyric acid). miRNA sequence alterations caused by juxtaposition of miR promoter/enhancer next to a protein encoding gene or vice versa, juxtaposition of one miRNA with another miRNA, fusion of one miRNA, and disruption of miRNA–PCG interaction have been reported in human cancers. Furthermore, the role of miRNAs in cell cycle regulation has been shown to be critical for protection against tumor formation. For example, suppression of let-7 miRNA, which controls the timing of cell cycle exit, leads to increased cell division and tumor growth in the lung tissue. Several groups have reported both overexpression and down-regulation of a number of miRNAs in human cancers using microarrays. These miRNA expression profiles serve as signatures to determine not only the stages of a cancer but also a potential therapeutic strategy.

The most abundant miRNA currently known in the liver, miR-122, is involved in cellular stress response, hepatocarcinogenesis, and inhibition of HCV replication. miR-122 down-regulation correlated with hepatocarcinogenesis in HCC developed in male Fisher rats fed a folic acid, methionine, and choline-deficient diet. An miRNA profile obtained from microarray studies showed up-regulation of let-7a, miR-21, miR-23, miR-130, miR-190, and miR-17-92 gene families in hepatomas. These findings suggest that down-regulation of miR-122 could be a potential biomarker for liver cancers. Another study revealed that miR-122a modulates cyclin G1 expression and increased cyclin G1 ex-
pression correlated with a higher incidence of primary liver carcinomas.193

The expression of miRNAs examined by microarray profiling found that miR-21 was highly overexpressed in HCC tumors and cell lines. Inhibition of miR-21 in cultured HCC cells increased expression of the PTEN tumor suppressor and decreased tumor cell proliferation, migration, and invasion; in contrast, enhanced miR-21 expression showed the opposite effect. Thus, PTEN is a direct target of miR-21. Moreover, the modulation of miR-21 also altered focal adhesion kinase phosphorylation and expression of matrix metalloproteases 2 and 9, downstream mediators of PTEN.194

Analysis of genomic sequence coding for the precursors of 59 microRNA genes in 96 HCC tissues and in eight liver cancer–derived cell lines to investigate whether germline mutations or natural polymorphisms contribute to liver cancer found four variations in three microRNAs (miR-106b, miR-192, and let-7a-2) in four HCC tissues, but no sequence variation was observed in any of the cell lines. The corresponding adjacent noncancerous tissues of those four specimens also carried the sequence variations. These data suggest that mutation in pre-miRNA is a rare event in HCC.195

RT-PCR was used to profile 200 precursor and mature miRNAs from 43 pairs of HCC and 28 pairs of benign liver specimens. miR-199a, miR-21, and miR-301 were differentially expressed in the tumor compared with adjacent benign liver. On the other hand, large number of mature and precursor miRNAs were up-regulated in the adjacent benign liver specimens. Further comparison of miRNA expression profile in the HCC tumors with patient’s survival time showed that a set of 19 miRNAs, involved in biological processes such as cell division, mitosis, and G1-S transition, significantly correlated with disease outcome.196 In a similar study, microarray studies were performed to identify microRNAs associated with HCC metastasis.197 A set of 20 miRNAs that correlated with patient survival were identified. These miRNAs may be useful to screen patients to identify those with a high likelihood of developing metastases/reoccurrence.

Future Perspectives

HCC is a complex disease with multiple underlying pathogenic mechanisms caused by a variety of risk factors. The lack of good molecular markers for HCC diagnosis and treatment assessment has posed a major challenge in health care. As discussed here, the expression of a large number of genes, proteins, and other molecules belonging to diverse cellular processes and pathways are altered in HCC. Therefore, no single test or set of tests is sufficient to provide an accurate assessment on hepatic tumor burden for every clinical situation. Molecules of each of the signaling pathways discussed are targets for gene therapy. Some of them are being used in clinical settings, others in clinical trials (reviewed by Tommasi et al.198). One of the objectives of global-scale studies for liver cancer is to determine the factors that contribute to the progression of cancer from normal tissue to metastasis. For this, a thorough understanding of the genotype–phenotype relationships based on environmental risk factors and the host’s genetic and hereditary traits is essential. The variability in the prognosis of individuals with HCC suggests that HCC may comprise several distinct biological phenotypes.88 These phenotypes may result from activation of different oncogenic pathways during tumorigenesis and/or from a different cell of origin. Molecular profiling of genes, proteins, and other molecules is aimed at deciphering the genotype–phenotype relationship with the goal of developing new therapies for human disease. Discoveries from such global studies may be helpful in developing personalized medicine. Omics technologies are expanding rapidly, and these new analytical strategies—combined with more efficient tissue procurement, protein isolation, and separation methods, as well as development of data analysis tools—are expected to increase our ability to detect novel biomarkers for the diagnosis, prognosis, and treatment of HCC.

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