Molecular Biology of Liver Cancer

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1 Introduction 207

2 Molecular Hallmarks of Hepatocellular Carcinoma 208
2.1 Genome Instability and Mutations 209
2.2 Sustaining Proliferative Signaling 211
2.3 Evading Growth Suppressors 212
2.4 Resisting Cell Death 213
2.5 Enabling Replicative Immortality 213
2.6 Inducing Angiogenesis 214
2.7 Activating Invasion and Metastasis 214
2.8 Reprogramming Energy Metabolism 215
2.9 Tumor-Promoting Inflammation 216
2.10 Evading Immune Destruction 217

3 Genome-Wide Changes in Hepatocellular Carcinoma 219

4 microRNA Profiling of Hepatocellular Carcinoma 222

5 Epigenetic Mechanisms 222

6 Concluding Remarks 222

References 228

Keywords

HBV
A small DNA virus that infects hepatocytes in the liver, causing acute or chronic hepatitis.

HCV
A small RNA virus that infects hepatocytes in the liver, causing acute or chronic hepatitis.

Genome-wide expression
An experimental approach for the identification of disease-specific gene expression profiles.

Gene signatures
Expression pattern of a set of genes associated with a clinical subtype of a disease.

PI3K/AKT pathway
A signaling pathway involved in cell survival that is hyperactivated in various cancers, including liver cancer.

MAPK/ERK pathway
A kinase protein cascade involved in cell survival that is upregulated in various cancers, including liver cancer.

p53
A tumor suppressor protein that is inactivated by mutation in cancers, including liver cancer.

hTERT
Human telomerase reverse transcriptase that is inactivated by mutation in cancers, including liver cancer.

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related mortality worldwide. Recent advances in the molecular profiling of HCC emphasize its intratumoral heterogeneity and reveal how cellular pathways are altered in favor of tumor progression. Malignant transformation of primary liver cancer is achieved through the acquisition of cancer hallmark capabilities that promote the uncontrolled proliferation of hepatocytes. In this review, the characteristics and acquired capabilities of human primary liver cancer, based on the HCC-specific genetic and epigenetic alterations, are described and discussed.

1 Introduction

Primary liver cancer is the fifth most frequently diagnosed cancer in men, and the seventh in women. However, due to its aggressive behavior and resistance to conventional therapies, liver cancer is the second most frequent cause of cancer death in men, and the sixth in women [1]. In 2008,

a total of 748 300 new liver cancer cases and 695 900 cancer deaths were reported worldwide [2]. Hepatocellular carcinoma (HCC) is the major histological subtype of primary liver cancers, accounting for about 80% of the total liver cancer cases worldwide [3].

The major risk factors for HCC are hepatitis B virus (HBV), hepatitis C virus (HCV) infections, alcohol, and aflatoxin B1 exposure. The development of HCC is a multistep process, where hepatic injury first leads to chronic liver disease, after which continuous inflammation results in cycles of cell death and hepatocyte regeneration. The subsequent expansion of dysplastic nodules, along with telomerase reactivation and increased genomic instability, is followed by malignant transformation [4]. The integration of HBV DNA into the host genome is frequent in HCC, whereas the RNA virus HCV leads to malignant transformation through oxidative stress and a proinflammatory response induced by viral proteins [5–7]. In addition, exposure to exogenous (aflatoxins) or endogenous (toxic metabolites such as steroids and cholesterol and metal ions such as copper, iron, nickel) hepatotoxic factors leads to liver injury and provokes an increase in the proliferating fraction of hepatocytes [8–12]. Therefore, chronic liver regeneration may itself be a source of spontaneous gene mutations leading to HCC [13].

The current classification system (Barcelona Clinic Liver Cancer; BCLC) considers HCC in five stages: very early; early; intermediate; advanced; and end-stage according to the tumor size, nodule number, vascular invasion, metastasis, and liver function [14]. The treatment strategy is determined based on the stage of the patient. Patients with very early and early HCC are treated with resection, liver transplantation or partial hepatectomy, while patients with intermediate HCC are treated with chemoembolization [15]. Unfortunately, however, patients are often diagnosed at an advanced stage, when these treatments cannot be applied. Although chemotherapy is the only treatment option for patients with advanced-stage HCC, patients with the same BCLC stage can still have variable responses due to high genomic heterogeneity of HCC [16, 17]. Therefore, a molecular level classification is necessary for effective diagnosis and personalized treatment options. Currently, genome-wide expression analysis, exome sequencing and microRNA (miRNA) profiling techniques are being used for the efficient molecular classification of HCCs.

Sorafenib (Nexavar; BAY43-9006), a multikinase inhibitor, is the only FDA-approved molecular-targeted agent for the treatment of patients with advanced HCC [18–20]. Sorafenib inhibits Raf, vascular endothelial growth factor receptor (VEGFR) kinase and platelet-derived growth factor receptor (PDGFR) kinase, and thereby suppresses cell proliferation and angiogenesis. In Phase III randomized controlled trials, sorafenib showed an overall survival benefit of three months [15, 21].

Primary liver cancer is a major public health problem, which requires in-depth molecular analysis in order to discover optimal targeted therapeutics. Due to the presence of genomic variations, custom-designed therapies based on the present understanding of the molecular biology of liver cancer will be indispensable for the treatment of HCC in the future.

2 Molecular Hallmarks of Hepatocellular Carcinoma

Malignant transformation requires the cancer cells to acquire several

growth-promoting characteristics in order to become tumorigenic and, eventually, malignant. Hepatocellular cancers derive from initially quiescent hepatocytes, the growth of which is tightly controlled. The appearance of tumor in this highly controlled microenvironment suggests that the initial HCC cells acquire phenotypic hallmarks of cancer, as described by Hanahan and Weinberg. In this case, six core and two emerging cancer hallmark capabilities have been suggested, namely: sustaining proliferative signaling; evading growth suppressors; resisting cell death; enabling replicative immortality; inducing angiogenesis; and activating invasion and metastasis, along with deregulating cellular energetics and avoiding immune destruction [22]. The acquisition of these capabilities is facilitated by two enabling characteristics, genome instability and tumor-promoting inflammation during the stepwise progression of HCC.

2.1 Genome Instability and Mutations

Tumorigenesis is a multistep process that initiates from dysplastic lesions which accumulate somatically acquired mutations and genomic instability [23]. Genomic instability, including chromosomal rearrangements and point mutations, is an enabling characteristic of multistep tumorigenesis and underlies the acquisition of hallmark capabilities [22].

HCC harbors chromosomal gains at 1q, 5, 6p, 7, 8q, 17q, and 20, and chromosomal losses at 1p, 4q, 6q, 8p, 13q, 16, 17p, and 21 [24, 25]. The gain of 1q and the loss of 1p and 17p are associated with early stages of HCC, while the gain of 6p, 5q, and 8q and loss of 4q and 8p are associated with advanced-stage HCC [26, 27]. Moreover, the integration of HBV DNA within or upstream of the telomerase reverse transcriptase (*TERT*) gene in the host genome was observed in patients with HBV-related HCCs [28, 29]. DNA-damaging reactive oxygen species (ROS) can also contribute to genomic instability [30, 31].

The *TERT* gene is frequently mutated in HCC, allowing the cells to gain replicative immortality. *TERT* promoter mutations in 44% of 61 HCC patients were shown to be associated with the early stages of HCC, independent of any viral infection, gender, age, and ethnicity [32]. *TP53* is the second most frequently mutated tumor suppressor gene in HCC, and is associated with a poor prognosis [33]. In HCCs, mutation rates are 50% and 20% with or without aflatoxin exposure, respectively [34]. A hot-spot mutation at codon 249 (R249S) is specific to AFB1 exposure in 36% of tumors from Africa and in 32% of tumors from China [35, 36]. Aberrant activation of the Wnt/β-catenin signaling pathway, which plays a key role in hepatocarcinogenesis, is achieved by activating mutations of β-catenin (CTNNB1) and inactivating mutations or loss of heterozygosity (LOH) of AXIN1 [24, 37–39]. Although mutations of the retinoblastoma gene (RB1) are rare, RB is frequently inactivated in HCC through the LOH of 13q, proteosomal degradation, or aberrant cyclin-dependent kinase activity [40–42]. The cyclin-dependent kinase inhibitor 2A gene (p16), which acts as a tumor suppressor downstream of p53 and Rb, is frequently inactivated in HCC [43, 44].

Mutations in the downstream elements of receptor tyrosine kinase (RTK) signaling are also observed in HCC. The inactivating mutations of the tumor suppressor gene phosphatase and tensin homolog (PTEN), which is a negative regulator of the phosphatidylinositol 3-kinase (PI3K)/Akt survival pathway, are observed in HCC,

Tab. 1 Molecular alterations of critical genes in HCC.

Cellular process	*Molecule*	*Alteration in HCC*	*Acquired capability*
Growth factor signaling	EGF/EGFR	Upregulation	Sustaining proliferative signaling
	HGF/MET	Upregulation	
	IGF/IGFR	Overexpression	Resisting cell death
	VEGF/VEGFR	Upregulation	Inducing angiogenesis
	PDGF/PDGFR	Upregulation	
	FGF/FGFR	Upregulation	
Cell cycle regulation	TP53	Inactivating mutation/LOH	Sustaining proliferative signaling Evading growth suppressors
	RB1	Inactivating mutation/LOH	
	c-myc	Overexpression	
	p16 (CDKN2A)	Inactivating mutation/hypermethylation	
	Cyclin D1	Overexpression	
	IRF2	Inactivating mutation	
Ras/RAF pathway	RAS	Activating mutation	Sustaining proliferative signaling Evading growth suppressors Inducing angiogenesis Activating invasion and metastasis
	RPS6KA3	Inactivating mutation	
PI3K/AKT pathway	PI3K-alpha (PIK3CA)	Activating mutation	Sustaining proliferative signaling Evading growth suppressors Inducing angiogenesis Activating invasion and metastasis Reprogramming energy metabolism
	PTEN	Inactivating mutation/LOH	
	AKT	Constitutive activation	
	mTORC1	Upregulation	
JAK/Stat pathway	Stat	Constitutive activation	Tumor-promoting inflammation
	SOCS1, SOCS3	Downregulation	
NF-κB pathway	NF-κB	Constitutive activation	Sustaining proliferative signaling Resisting cell death Tumor-promoting inflammation
Wnt/β-catenin pathway	β-Catenin (CTNNB1)	Activating mutation/overexpression	Sustaining proliferative signaling Tumor-promoting inflammation
	AXIN1, AXIN2	Inactivating mutation/LOH	
	APC	Inactivating mutation	

(*continued overleaf*)

Tab. 1 (*Continued.*)

Cellular process	*Molecule*	*Alteration in HCC*	*Acquired capability*
Hedgehog pathway	SHH	Overexpression	Reprogramming energy metabolism
	SMO	Overexpression	
	HHIP	LOH, hypermethylation	
Histone modification	DNMT1, 3A, 3B	Upregulation	Sustaining proliferative signaling Tumor promoting inflammation
	EZH2	Overexpression	
	ARID1, ARID2	Inactivating mutation	
Apoptosis	Fas	Downregulation	Resisting cell death
	FasL	Upregulation	
	DR5	Downregulation	
Angiogenesis	Angiopoietin	Upregulation	Inducing angiogenesis
	Tie-2	Upregulation	
Immunity	Glypican-3	Upregulation	Evading immune destruction

LOH: Loss of heterozygosity.

but not very frequently [45–49]. Ras proto-oncogenes (H-*ras*, K-*ras*, N-*ras*) are also rarely mutated and activated in HCC [43, 50–52]. Runt-related transcription factor (RUNX) family genes induce a senescence-like growth arrest in response to oncogenic Ras. The expression of one of the members of this family, RUNX3, is decreased in more than 50% of HCC cases [53, 54], its downregulation being associated with escape from apoptosis and sustained growth [55, 56]. Recently, the role of RUNX3 in mediating angiogenesis and epithelial-mesenchymal transition (EMT) was also demonstrated [57]. Thus, the loss of RUNX expression may explain the rarity of Ras mutations in these cancers.

Less-frequent inactivating tumor-suppressing mutations of P14, IGFR2, KLF6, hepatocyte nuclear factor (HNF) 1α, SMAD2, SMAD4 and LKB1/STK11, and less-frequent activating oncogenic mutations of EGFR, Erb2 and PIK3CA have also been reported in HCC [58, 59]. Molecular alterations of critical genes and their contribution to the acquisition of hallmark capabilities are listed in Table 1.

2.2 Sustaining Proliferative Signaling

Constitutive activation of survival pathways, the inactivation of tumor suppressors TP53, Rb and p16, the overexpression of c-myc and cyclin D1, the epigenetic silencing of p16INK4, and the overexpression of E2F family members promote cell cycle progression and a sustained proliferation of HCC cells.

Growth factors and their corresponding activated tyrosine kinase receptors such as EGF-EGFR, IGF-IGFR and HGF-MET transmit the proliferation signal through Ras/Raf/MEK/ERK and PI3K/AKT/mTOR

pathways. Constitutive activation of these pathways are maintained through the overexpression of MET and EGFR and the inactivation or downregulation of the negative regulators PTEN and RASSF1A of PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways, respectively. The identification of potential driver genes in human liver carcinoma by genome-wide screening revealed genes with specific signaling pathways such as PI3K/Akt/mTOR, AMP-activated protein kinase (AMPK) and EGFR [60]. Cell proliferation in HCV-positive HCC is also associated with Myc and AKT activation [61]. Moreover, it is known that the overexpression of E2F family members, which are responsible for the transcription of genes involved in cell cycle and proliferation, inhibits c-Myc-driven apoptosis through the PI3K/AKT/mTOR pathway [62, 63].

Additionally, large-scale analyses with high numbers of HCC samples revealed proliferation gene signatures. Chen *et al*., in 2002, showed a high expression of a "proliferation cluster" comprised of genes required for cell cycle progression in 102 primary HCC tumor samples and in 10 HCC cell lines [64]. Ribosomal protein genes were highly expressed, as expected in cells with unlimited cell growth. Upregulated genes were related to DNA replication and G_2/M progression. In another study of 91 human primary HCCs, a proliferation gene signature (containing PCNA, Bub3, MCM2, MCM6, MCM7, cyclinA2, cyclinB1, CKS2, and CDK4) was able to distinguish between two groups of HCC patients with a distinct prognosis [65].

2.3 Evading Growth Suppressors

The inactivation of pRB, p53 and p16, and the overexpression of c-myc and cyclin D1 confer growth advantages in HCC. Activation of the MYC transcription signature is strongly associated with the malignant conversion of preneoplastic liver nodules, while the inactivation of MYC in invasive HCCs leads to a sustained tumor regression as well as proliferation arrest, differentiation and apoptosis of malignant cells [66, 67].

The HCV core protein induces promoter hypermethylation and a downregulation of p16 expression, and subsequently induces Rb phosphorylation that leads to the activation of E2F1 that, in turn, stimulates cell growth [68]. This mechanism is also exploited to overcome stress-induced premature senescence in the presence of HCV-induced oxidative stress [69]. Furthermore, HCV core protein upregulates DNA methyltransferases 1 and 3b, and induces the promoter hypermethylation of retinoic acid receptor-β2 (RAR-β2). This mechanism leads to an escape from RB/E2F-related growth arrest induced by all-*trans* retinoic acid [70].

Upregulation of the oncoprotein gankyrin enhances the transcriptional activity of β-catenin, which in turn transcriptionally activates gankyrin by a positive feedback loop [71]. Gankyrin is highly expressed in HCC, and its overexpression mediates the degradation of the tumor suppressor proteins Rb and p53 and thereby accelerates cell cycle progression.

Growth arrest and DNA damage 45G (GADD45G) is commonly downregulated in oncogene-transformed HCC [72]. The ectopic expression of GADD45G induces senescence in HCC through a repression of the Jak/Stat3 pathway, independently of p53, p16INK4a and Rb. The expression of constitutively activated Stat3 or human telomerase reverse transcriptase (hTERT) reverts GADD45G-induced senescence.

Transforming growth factor-beta (TGF-β) signaling has a growth-suppressive role in

the early stages of HCC. Resistance to TGF-β signaling-mediated growth inhibition is a frequent event during the malignant transformation of hepatocytes. In the advanced stages of HCC, TGF-β is secreted by stromal cells in the tumor microenvironment, and therefore induces apoptosis in hepatocytes. However, downregulation of the receptors of TGF-β and upregulation of the EGFR and MEK/ERK pathways confers resistance to TGF-β-induced cell death in liver tumor cells [73–79].

2.4 Resisting Cell Death

The presence of elevated levels of growth factors such as IGF, the upregulation of anti-apoptotic pathways such as NF-κB pathway, the downregulation of death receptors such as DR5 and Fas, and mutations in the tumor suppressor genes such as p53, contribute to an evasion of apoptosis in many cancers, including liver cancer. The IGF/IGFR signaling pathway that is constitutively activated in 20% of HCC via IGF2 or IGFR1 overexpression, regulates proliferation, motility, invasion, and the inhibition of apoptosis. This is correlated with the stage, metastasis and survival of HCC [80–84]. The IGF pathway activates cell-survival pathways PI3K/AKT and RAF/MEK/ERK and provides a mechanism by which to evade apoptosis [80].

Mutations in the tumor suppressor gene *p53* cause a loss of apoptotic response. Large-scale gene expression analysis showed that cell cycle-related genes (*CCNG2*, *BZAP45*) and cell proliferation-related genes (*SSR1*, *ANXA2*, *S100A10*, and *PTMA*) were overexpressed in mutant-*p53* tumors compared to wild-type-*p53* tumors in HCV-related HCC [85].

Tumor necrosis factor (TNF) activates the antiapoptotic NF-κB pathway, the pro-apoptotic caspase-cascade, and JNK kinases in HCC. Inhibition of NF-κB by NEMO results in an upregulation of the death receptor DR5, whose ligand (TRAIL) is predominantly expressed by natural killer (NK) cells and is essentially involved in liver injury in NEMO-deficient hepatocytes [86]. Furthermore, Caspase-8 is frequently inactivated in HCC and therefore interferes with the proapoptotic caspase-cascade [87]. The downregulation of Fas expression, upregulation of its ligand (FasL) expression in hepatocytes, and the elevation of serum soluble Fas levels were also identified as critical players of evasion from immune surveillance, and hepatic carcinogenesis [88].

2.5 Enabling Replicative Immortality

It is known that the reactivation of telomerase maintains telomere length and replicative immortality during cirrhosis, and therefore leads to HCC progression [89, 90]. Not only telomere dysfunction but also oncogene activation, persistent DNA damage and ROS-induced oxidative stress can cause permanent cell cycle arrest, known as senescence [31, 91]. The ability to bypass senescence is a characteristic of liver cancer cells in gaining replicative immortality.

TGF-β, Ras, Raf, Mos, Mek, Myc, E2F, Stat5, Cyclin E and PTEN are all key players of oncogene-induced senescence [92]. The reactivation of telomerase and inactivation of p53, p15, p16 and p21 may play critical roles in the bypass of senescence and maintenance of immortality [91]. Indeed, the expression of human hTERT was able to revert GADD455-induced senescence in HCC [72], while an upregulation of hTERT expression by low-dose cisplatin contributed to cell death resistance in a

HCC cell line; this resistance was reverted by an inhibition of hTERT [93]. Two independent mutations were identified within the core promoter of TERT; these mutations increased transcriptional activity from the TERT promoter by two- to four-fold, and were shown to occur frequently in HCC [94]. A recurrent integration of HBV into the promoter of the TERT gene was correlated with increased TERT expression in HBV-related HCC patient samples [95]. Furthermore, a recent study showed that HCCs expressing "stemness"-related proteins (K19, EpCAM, CD133) have increased telomere length, an increased expression of hTERT and shelterin complex proteins (TRF1, TRF2, TIN2, POT1, TPP1, RAP1), and an increased chromosomal instability compared to HCCs without these markers [96]. Hence, high telomerase activity and long telomeres in HCCs are associated with an aggressive behavior and a poor prognosis [95, 97]. It was reported recently that there is a major shift from senescence-associated gene expression to immortality-associated gene expression during the transition from dysplasia to early HCC lesions. Moreover, a senescence bypass signature was able to differentiate HCC from cirrhosis [98]. Therefore, targeting senescence in liver cancer treatment can be considered as an alternative mechanism in addition to classical chemotherapeutic agents [99].

2.6 Inducing Angiogenesis

Angiogenesis and neovascularization involve interactions between tumor cells, vascular endothelial cells (VECs) and their supporting pericytes in order to supply oxygen and nutrients to the growing tumor [30]. In HCC, the balance between proangiogenic and antiangiogenic factors is disrupted due to an excess secretion of angiogenic factors by endothelial cells and pericytes in the tumor microenvironment [100]. Angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiopoietin-2, Tie-2 are upregulated in HCC. This process induces angiogenic signaling through activation of the RAF/MEK/ERK, PI3K/AKT/mTOR, JAK/Stat, and HGF/MET pathways [101–106].

VEGF and its receptors VEGFR-1 and VEGFR-2 are overexpressed in HCC, and are associated with aggressiveness and a poor prognosis [107–111]. The HBV × antigen also upregulates VEGFR-3 [112]. VEGF acts synergistically with FGF, the overexpression of which is correlated with HCC angiogenesis [113, 114].

Hypoxia, which occurs during fibrosis, cirrhosis and malignant transformation, enhances the proliferation, angiogenesis, metastasis, chemoresistance and radioresistance of HCC [100]. Hypoxia-induced factor 1 alpha (HIF-1α) promotes hepatocyte EMT through PI3K/Akt, TGF-β and β-catenin signaling, and this is associated with an enhanced metastatic potential and a poor prognosis in HCC [115–117]. Bone morphogenetic protein 4 (BMP-4) is also induced in hypoxic conditions and promotes vasculogenesis and tumor progression in HCC [118].

2.7 Activating Invasion and Metastasis

Cell detachment is an early step of tumor invasion, requiring alterations to the adhesive properties of cancer cells in general. Therefore, EMT is critical for activating the invasion and metastasis of HCC. The upregulation and activation of Twist, Snail, Slug, Zeb1/2 and Vimentin, and

the downregulation of E-cadherin and HNF-4α, frequently occur during EMT and correlate with a poor prognosis in liver cancer [119–122]. Furthermore, p53 regulates EMT through miR-200 family members and miR-192, which targets Zeb1 and Zeb2 [123]. HBx expression was shown to induce EMT by activating the PI3K/Akt/GSK-3β pathway, which stabilizes Snail and mediates integrin $\alpha_6\beta_1$ signaling, thus facilitating tumor invasion and metastasis during HCC progression [124, 125]. In HCV-positive HCC patients, the expression of a four-gene signature, including E-cadherin, inhibitor of DNA binding 2 (ID2), MMP9 and transcription factor 3 (TCF3), is correlated with poor prognosis [126].

Osteopontin is overexpressed in metastatic HBV-related HCC and invasion, and metastases are effectively blocked by an osteopontin-specific antibody both *in vitro* and *in vivo* [127]. This observation suggests that osteopontin can be considered as a diagnostic marker and a potential therapeutic target for HBV-related metastatic HCC [128, 129]. The tumor suppressor DLC1 and cytoskeletal protein RhoA are also involved in the prevention of dissemination and metastasis of human HCC cells in nude mice [130, 131]. Additionally, intratumoral hypoxia triggers the invasion and metastasis of HCC through an oncogenic HGF/MET signaling pathway [132]. A Met-regulated gene expression signature defines an aggressive subtype of HCC with an increased vascular invasion rate, microvessel density, and a decreased mean survival time of HCC patients [103].

2.8 Reprogramming Energy Metabolism

Cancer cells reprogram their energy metabolism so that they can use glucose to supply energy through aerobic glycolysis and glutamine to provide intermediates of the tricarboxylic acid (TCA) cycle [133]. In addition, autophagy enables fast-growing cells to break down cellular organelles, and this results in recycled catabolites that can be used for biosynthesis and energy metabolism [22]. mTOR, an evolutionarily conserved serine/threonine kinase located downstream in the PI3K/AKT pathway, also functions as a nutrition sensor to monitor cellular metabolism [134]. In the presence of sufficient energy and nutrients, active mTOR promotes translation and biosynthesis, and hence suppresses autophagy. In the absence of sufficient energy and nutrients, however, mTOR with downregulated activity leads to a reduction in biosynthesis and promotes autophagy [135]. The PI3K/AKT pathway also stimulates glucose uptake and metabolism for the continued growth and survival of cancer cells [136].

In contrast, glutamine uptake and metabolism is under the control of c-myc [137]. Elevated energy consumption and an addiction to mitochondrial glutaminolysis is dependent on the AMPK-related kinase 5 (ARK5) through oncogenic c-myc expression in HCC cells. ARK5 limits protein synthesis via an inhibition of mTORC1 and maintains the high respiratory capacity required for efficient glutamine metabolism [138]. Therefore, targeting cellular energy homeostasis represents a promising therapeutic strategy for HCC cells with a higher c-myc expression. AMPK, which is activated in response to reduced energy levels, promotes ATP production by increasing catabolism, and conserves ATP by switching off biosynthetic pathways. AMPK was found to be dysfunctional in patients with HCC, and low p-AMPK levels were correlated with aggressiveness and a poor prognosis [139].

Moreover, AMPK is also activated by the p53 targets, Sestrin1 and Sestrin2 [140]. p53 stimulates oxidative phosphorylation and reduces the rate of glycolysis through the upregulation of TP53-induced glycolysis and apoptosis regulator (TIGAR) [141]. Therefore, energy metabolism is shifted from mitochondrial respiration towards glycolysis by the loss of p53 in cancer cells.

During HCC development, metabolic remodeling from mitochondrial oxidation to glycolysis was assessed by a combined transcriptomics and metabolomics study in six subgroups of HCC tissues, as defined by Beyoğlu *et al*. [142]. HCC has lower levels of glucose and other metabolites (glycerol 3-phosphate, glycerol 2-phosphate, malate, alanine, and myoinositol) involved in energy production compared to healthy liver. Moreover, concentrations of certain saturated lipids are reduced in a subgroup of HCC cells associated with high serum alpha-fetoprotein (AFP) levels. This is consistent with the previous observations on the upregulation of lipid catabolism accompanied by elevated AFP expression. In another study, 28 metabolites and 169 genes were identified that were involved in energy metabolism associated with aggressive HCC [143]. Metabolic activities within the HCC microenvironment are also promoted by Hedgehog signaling activation by malignant hepatocytes. Hedgehog ligands produced by these cells stimulate glycolysis in the neighboring myofibroblasts, resulting in the release of myofibroblast-derived lactate that can be used as an energy source by the malignant hepatocytes [144].

Mutations that activate oncogenes (e.g., c-myc, HIF-1α, and PI3K/AKT) or inactivate tumor suppressors (e.g., p53, PTEN, TSC2, and LKB1) have been shown to contribute to metabolic alterations in various types of cancer [145]. Oncogene-altered energy metabolism presents a new class of target molecules for tumor therapy. Indeed, activating oxidative phosphorylation by a pyruvate dehydrogenase kinase inhibitor (dichloroacetate) overcame the sorafenib resistance of HCC, while a combination of sorafenib and dichloroacetate resulted in an elevated tumor regression compared to sorafenib alone [146]. Therefore, tumor bioenergetics can be further exploited for HCC therapy.

2.9 Tumor-Promoting Inflammation

The tissue microenvironment plays a critical role in HCC formation and development [100]. The HCC microenvironment is composed of cancer-associated fibroblasts (CAFs), invading inflammatory cells, endothelial cells, pericytes adjacent to the endothelial cells, hepatic stellate cells (HSCs), macrophages (Kupffer cells), dendritic cells (DCs), and stem/progenitor cells. The extracellular matrix (ECM) components, including collagen, fibronectin, laminin, glycosaminoglycans and proteoglycans, provide a supportive microenvironment for these cells [100, 147]. All of these components of the microenvironment interact with each other and produce growth factors, cytokines, chemokines and free radicals that contribute to liver fibrosis, and therefore also tumor initiation and progression. Overexpression of the highly negatively charged ECM protein osteopontin is associated with large tumor size, advanced tumor stage, capsular infiltration, vascular invasion, lymph node invasion, intrahepatic metastasis, early recurrence, and poor prognosis of HCC [148–154]. Furthermore, plasma osteopontin levels

were significantly higher in HCC patients, and thus can be considered as an early HCC marker, together with AFP [151, 155, 156].

During HBV- or HCV-infection-associated chronic liver disease, hepatocyte injury leads to inflammatory cell infiltration, where the host immune cells destroy virus-infected hepatocytes [157, 158]. The continuous inflammation results in a cycle of hepatocyte death and proliferation, leading to an increased genomic instability and mutations [4]. When stimulated with proinflammatory cytokines (IL-1β, TNF-α, and PDGF), Kupffer cells and HSCs produce osteopontin that plays an important role in inflammation, growth, invasion, metastasis, angiogenesis and inhibition of apoptosis [159–162]. TNF-α, produced by Kupffer cells and other immune cells, promotes tumor progression mainly through the NF-κB and Akt pathways [159].

Kupffer cells express and release the proinflammatory cytokine interleukin-6 (IL-6). The latter is one of the major mediators of inflammation, and activates the STAT3 pathway to mediate its signal through the gp130 protein. IL-6 also protects liver cells against apoptosis via the STAT3 pathway following viral infection or chemical ingestion [163, 164]; this mechanism is also exploited during tumor promotion [165]. Constitutively activated Stat3 protein maintains NF-κB activity in tumors by preventing the nuclear export of NF-κB complex through RelA acetylation [166]. RelA, in turn, maintains a persistent activation of STAT3 and IL-6 in the HCC microenvironment [167]. Therefore, a conditional knock-out of STAT3 expression impairs liver regeneration, whereas a tissue-specific knock-out of hepatic STAT3 effects glucose homeostasis and the induction of insulin resistance [168, 169].

Oncogenic β-catenin also triggers an inflammatory response, including activation of the NF-κB pathway in hepatocytes, which promotes aggressiveness of HCC in mice [170]. Elevated IL-6 levels and constitutively activated STAT3 has been frequently detected in HCC patients and in cell lines [171–176]. In addition, the Jak-Stat inhibitors SOCS1 and SOCS3 are downregulated in HCC via promoter hypermethylation [177]. Moreover, somatic gain-of-function mutations in the IL6ST gene (gp130), have been identified in inflammatory hepatocellular adenomas [178]. IL-6/STAT3 further activates several interleukins and growth factors.

A low-grade inflammatory response is induced upon lipid accumulation in obesity, which in turn increases IL-6 and TNF expression by adipose tissue and Kupffer cells [179, 180]. IL-6 and TNF signaling promotes the proliferation of damaged hepatocytes via an activation of JAK/STAT and AKT/ERK, respectively [159, 181].

A unique 17-gene immune response signature of the liver microenvironment could predict venous metastases, recurrence, and prognosis in HCC [182]. A global Th1- to Th2-like cytokine shift is associated with HCC metastasis, which is promoted through a shift toward anti-inflammatory/immune-suppressive responses.

2.10 Evading Immune Destruction

Liver cancer develops usually on top of chronic inflammation during fibrosis and cirrhosis. Yet, even though tumor cells exploit inflammation in favor of their growth, as mentioned above, they still need to escape from immune destruction. HCC cells evade immune destruction by

expressing immunosuppressive molecules such as PD-L1 and indolamine 2,3-dioxygenase (IDO), and also by secreting cytokines and chemokines such as IL-6, IL-10, TGF-β and VEGF [183]. The resulting immunosuppressive microenvironment is supported by the induction of regulatory dendritic cells (DCregs), regulatory T cells (Tregs), and tumor-associated macrophages (TAMs) and the suppression of DCs, effector T cells and NK cells.

Macrophages are the major infiltrating leukocytes, and are involved in both innate and adaptive immune responses [184]. During either HBV- or HCV-infection, a fibrotic and cirrhotic liver causes inflammatory cell infiltration due to the necrosis of hepatocytes. Therefore, the status of TAMs and other immune cells in the tumor microenvironment is closely associated with the suppression of antitumor immunity and progression of HCC. Polarizing inflammatory responses toward the preferential recruitment of Th2-type cells and Tregs rather than Th1-type cells promotes tumor immune evasion. While M2-type "alternatively activated" macrophages promote tumor progression, M1-type "classically activated" macrophages can exert antitumor activity by killing the tumor cells. Therefore, shifting the macrophage balance from tumor promotion by innate immunity-driven inflammation towards tumor surveillance by adaptive immune responses can be an effective therapeutic strategy [185].

Dendritic cells, which are important for chronic liver inflammation, express and present antigens to infiltrating cytotoxic T lymphocytes (CTLs). The dendritic cells express Glypican 3 (GPC3), the upregulation of which is associated with a poor prognosis in HCC [186]. Indeed, tumor-induced DCregs with hyperactivate Stat3 can facilitate tumor immune evasion independent of Stat3 hyperactivation status in tumors [187]. Additionally, a CTL-mediated immune response can be impaired by Kupffer cells through programmed death ligand 1 (PD-L1), and regulatory dendritic cells (CD14+ CTLA4+) through CTLA4-dependent IL-10 and IDO production, enabling immune evasion [188, 189].

Cytokine production by NK cells in chronic HCV infection is shifted towards the secretion of Th2-type cytokines, promoting an environment which is more permissive for HCV [190]. In chronic HCV patients, NK cells have a reduced cytotoxicity and interferon (IFN)-γ production, and secrete IL-10 and TGF-β; this results in the induction of Th2 cells and Tregs, maintaining immune evasion. Furthermore, many immune system-related genes, including SATB1, TNFRSF5, CTLA4, GITR, SIRPα, PD-L1 and ICOS, have altered expressions in HCC [85, 191, 192].

It has been shown that the only FDA-approved chemotherapeutic agent, sorafenib, also suppresses the proliferation and activation of NK cells in addition to malignant hepatocytes. Consequently, the reduced cytotoxicity of NK cells handicaps HCC patients during treatment by rendering the host more susceptible to tumor growth and metastasis [193]. Therefore, immunotherapeutic approaches activating NK cells can enhance the efficacy of sorafenib.

In light of above-described cellular mechanisms, the hallmarks of cancer are represented in parallel with the altered genes involved in the development of the multistep progression of liver cancer, in Fig. 1. This overall picture demonstrates that genome instability and mutations, along with sustaining proliferative signaling having the highest number of altered genes,

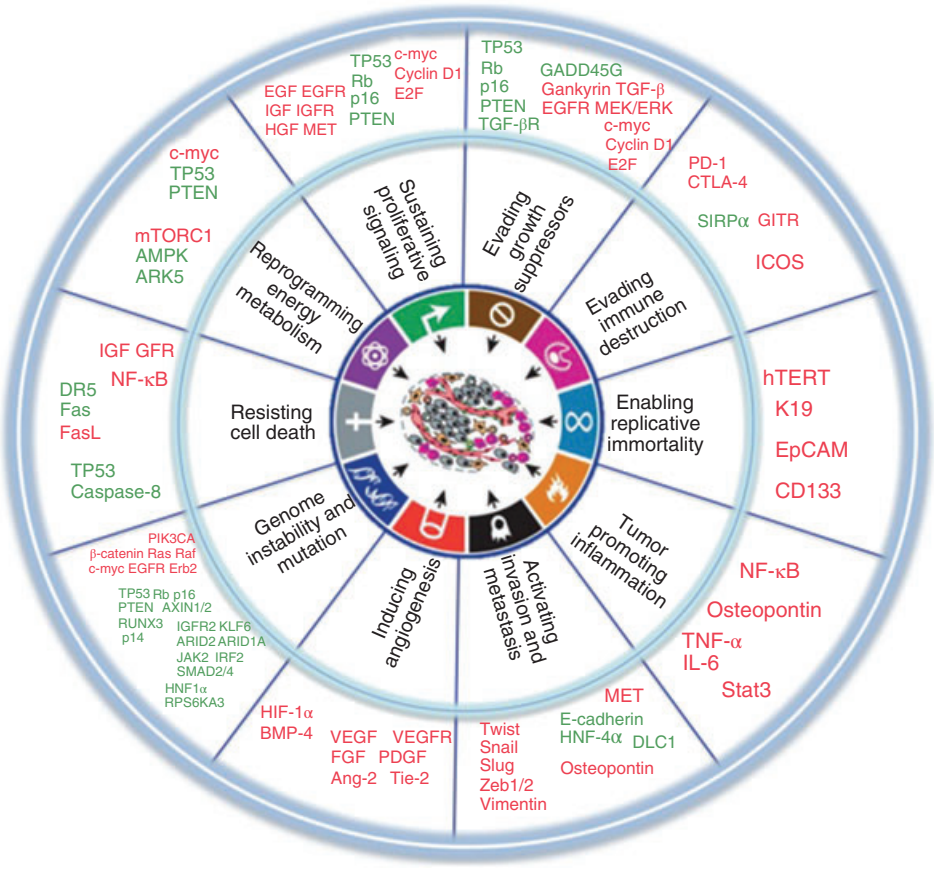

Fig. 1 Molecular hallmarks of hepatocellular carcinoma. Molecular alterations promote the hallmark capabilities through either activation/expression/upregulation are shown in red, while inactivation/loss/downregulation are shown in green. Modified with permission from Ref. [22].

are the most widely studied hallmarks in HCC. Yet, the new emerging capabilities of tumor cells should be further examined in this disease in order to identify novel genes associated with HCC malignancy.

3 Genome-Wide Changes in Hepatocellular Carcinoma

During the past decade, several high-throughput analyses on HCC samples have been reported. Microarray, whole-genome sequencing and exome sequencing studies will enlighten the intra-tumoral heterogeneity of HCCs from various etiologies and histological pathologies. These large-scale studies revealed novel HCC-related pathways or gene signatures that can distinguish subgroups of HCC based on etiology, molecular background, and histopathology. These signatures also predict survival, metastasis, and recurrence. Major findings of recent high-throughput analyses on HCC are presented in Table 2. Some of the novel

Tab. 2 Major findings of recent high-throughput analyses on HCC.

Classification of HCC based on altered gene expression	*Reference*
Highly expressed "proliferation cluster" genes in HCC	[64]
"Proliferation cluster" genes predict survival by 406 gene signature	[65]
HCC classified into two groups based on IFN-regulated and apoptosis-relevant genes	[194]
Identify 240 gene signature for low- to high-grade dysplastic nodules and HCC	[195]
Six subgroups of HCC related to genetic alterations	[43]
G1: IGFR1 activation, AKT activation, developmental imprinting	
G2: PIK3CA and TP53 mutations, AKT activation	
G3: TP53 mutation and overexpression of cell-cycle genes	
G4: a heterogeneous subgroup	
G5: β-catenin mutations, Wnt/β-catenin activation	
G6: G5 with satellite nodules, having higher activation of the Wnt pathway and low E-cadherin expression	
Four neoplastic stages of HCV-positive HCC	[196]
Control vs. cirrhosis: 8 gene signature	
Cirrhosis vs. dysplasia: 24 gene signature	
Dysplasia vs. early HCC: 93 gene signature	
Early vs. advanced HCC: 9 gene signature	
Subclasses of HCC with different genetic backgrounds	[197]
c-Myc induced, 6p/1q-amplified, 17q-amplified	
Five classes of HCV-positive HCC	[111]
CTNNB1 class: β-catenin mutations	
Proliferation class: IGF1R activation and RPS6 phosphorylation	
IFN-related class: a novel class defined by polysomy of chromosome 7	
Three molecular subclasses of HCC	[61]
S1: Wnt/β-catenin activation	
S2: proliferation with Myc and AKT activation and IFN repression	
S3: tumor size, differentiation, and serum AFP levels	
Cirrhosis vs. HCC	[98]
Immortality and senescent signature: 15 immortality gene	
Classification of HCC based on chromosomal imbalance	
Early vs. advanced stages of HCC	[198]
Three HBV-positive HCC subgroups predict survival	[199]
Classification of HCC based on different etiologies	
Invasiveness gene signatures of HBV- or HCV-associated HCC	[200]
HBV- or HCV-positivity	[201]
HBV-positive HCC: 31 genes relating to signal transduction, transcription, metastasis	
HCV-positive HCC: 52 genes related to detoxification and immune response	
HBV-positive HCC vs. non-tumor liver tissues	[202]
44 gene signature	
Early vs. late stages of HBV-positive HCC	[203]
65 gene signature	
Susceptibility to HBV-positive HCC	[204]
1p36.22 locus, and KIF1B-, UBE4B-, PGD-related pathways	
Susceptibility to HCV-positive HCC	[205]
Common variants within the DEPDC5 locus on chromosome 22	

(*continued overleaf*)

Tab. 2 (*Continued.*)

Classification of HCC based on altered gene expression	*Reference*
HCV-positive HCC	[206]
Strong association of a locus in the 5′ flanking region of MICA, which leads to activation of natural killer cells and CD8+ T cells	
Molecular profile of specific gene alterations	
HCV-positive HCC with mutant-p53 vs. wild-type-p53	[85]
Overexpressed: cell cycle- and cell proliferation-related genes	
Underexpressed: immune system-related genes	
Myc signature in cirrhosis vs. nodules vs. HCC	[66]
Met knock-out signature: HGF/Met activation associated with poor survival	[103]
RB knock-out signature: increased proliferation and RB/E2F activity	[207]
TGF-ß knock-out signature: invasive phenotype and increased tumor recurrence	[208]
Identification of genetic alterations	
Somatic substitutions in HCV-positive HCC: T > C/A > G and C > T/G > A 11 731 mutations, including TP53, AXIN1, ADAM22, JAK2, KHDRBS2, NEK8, and TRRAP	[209]
Frequent mutations in HCV-associated HCC, including CTNNB1, TP53, ARID2, DMXL1, and NLP1	[210]
ARID2 as a tumor suppressor gene in HCV-associated HCC	
Cirrhotic vs. non-cirrhotic HCC: G > T and C > A transversions are more frequent in tumors from non-cirrhotic liver	[24]
New recurrent alterations in ARID1A, RPS6KA3, NFE2L2, and IRF2	
Identification of epigenetic alterations	
Predict survival: aberrant DNA methylation signature at promoter sites	[211]
High frequency chromatin regulating gene mutation	[29]
Mutation in ARID2	
Identification of potential tumor driver mutations	
50 potential genes with specific signaling pathways (mTOR, AMPK, and EGFR)	[60]
3 tumor mutations (CCNG1, P62, and an indel/fusion gene) from sequencing of 3 HCC nodules from 1 HBV-positive HCC patient	[212]
13 potential tumor suppressor genes, including XPO4	[213]
Prediction of metastasis from gene signatures	
153 gene signature in HBV-positive HCC	[127]
Osteopontin overexpression	
17 gene stromal tissue signature to predict metastasis	[182]
HBV-positive HCC patients with portal vein tumor thrombosis	[214]
Transversions: C : G > A : T and T : A > A : T	
Mutation in ARID1A	
Prediction of recurrence from gene signatures	
12 gene signature	[215]
Early intrahepatic recurrence	[216]
57 gene signature	[217]
Early recurrence in HBV-positive HCC	[218]
Late recurrence from stromal tissues	[219]
Molecular profile of poorly differentiated cells	
Hepatoblast-like subclass with AP1 activation associated with poor prognosis	[220]
Progenitor-like class with EPCAM and AFP in HBV-positive HCC EPCAM-/AFP+ are associated with poor prognosis	[221]

important genes associated with HCC are also included in the table, along with their respective study.

4 microRNA Profiling of Hepatocellular Carcinoma

Recent findings highlight the importance of microRNAs in mediating the acquired capabilities of HCC. Comprehensive analyses of microRNA expression patterns have revealed a differential expression of miRNAs in metastatic HCC and non-metastatic HCC compared to healthy liver (Table 3). Hence, miRNAs can be utilized as prognostic markers in HCC patients with various clinical phenotypes. miR-26a, the expression of which is reduced in HCC, inhibits angiogenesis by downregulating VEGFA through PIK3C2α/Akt/HIF-1α, and suppresses growth and metastasis through IL-6/Stat3 signaling [222, 223]. miRNA replacement therapy, where miR-26a is administered in a mouse model of HCC, inhibits cancer cell proliferation and induces apoptosis [224]. The delivery of downregulated miRNAs that are highly expressed and therefore tolerated in normal liver inhibits tumorigenicity without toxicity; hence, this approach may be a valuable strategy for miRNA-mediated HCC therapies.

5 Epigenetic Mechanisms

The epigenetic regulation of gene expression involves DNA methylation, post-translational histone modifications, and changes in the expression profiles of chromatin-modifying enzymes, which are highly deregulated in cancers, including HCC [253].

Epigenetic alterations in HCC include global DNA hypomethylation, gene-specific DNA hypermethylation of Rb, E-cadherin, RASSF1A and p16, gene-specific DNA hypomethylation of Vimentin, uPA and CD147, upregulation of DNA methyltransferases DNMT1, DNMT3A, and DNMT3B, and altered histone modification patterns of H3K9 and H3K27 through deregulation of the histone-modifying enzymes HDAC1/2/3, SIRT1, EZH2, and ARID2 [24, 29, 210, 254–260].

The histone methyltransferase EZH2 is overexpressed in HCC and contributes to the epigenetic silencing of target genes that regulate cancer cell growth and survival. Sorafenib was shown to downregulate EZH2 protein levels by accelerating its proteasome-mediated degradation in hepatoma cells, and thereby altering the HCC epigenome by reducing H3K27 trimethylation [261]. The overexpression of EZH2 reverses sorafenib-induced cell cycle arrest, and apoptosis. This epigenetics-based study revealed a novel combinational therapy approach, where an inhibition of EZH2 can be used to increase the sensitivity of HCC cells to chemotherapeutic agents.

All hallmarks of HCC are under the control of epigenetic mechanisms. In return, an altered metabolism in HCC cells can determine the availability of metabolites that are necessary for the functioning of epigenetic modifiers and thereby regulate the cancer epigenome [262]. Aberrant activities of epigenetic regulators and gene-specific methylation alterations in HCC are listed in Tables 4 and 5.

6 Concluding Remarks

HCC, as one of the leading causes of cancer-related death, is mostly a viral

Tab. 3 Alterations in microRNAs and their mechanisms of promoting HCC progression.

microRNA	*Mechanism*	*Alteration in HCC*	*Reference(s)*
miR-101	Targets EZH2 and inhibits HCC progression	Downregulation	[225]
miR-122	Sensitize HCC cells to chemotherapeutic drugs by downregulating MDR related genes	Downregulation	[226]
miR-124	Targets PIK3CA, suppresses PI3K/AKT pathway Suppresses the HCC growth through targeting STAT3 Transient inhibition of HNF4α initiates HCC through a microRNA-inflammatory feedback loop of miR-124, IL6R, STAT3, miR-24, and miR-629	Downregulation	[227–229]
miR-139	Promotes cell proliferation and invasion through the WNT/TCF-4 pathway	Downregulation	[230]
miR-140-5p	Suppresses cell proliferation and metastasis by targeting TGFBR1 and FGF9	Downregulation	[231]
miR-148a	Suppresses EMT and metastasis by targeting Met/Snail Reduces HPIP, represses AKT and ERK and inhibits mTOR through AKT/ERK/FOXO4/ATF5	Downregulation	[232, 233]
miR-155	Targets APC, promotes hepatocyte proliferation and tumorigenesis by activating Wnt signaling	Overexpression	[234]
miR-17	Inhibits cell migration and invasion via suppression of MMP-3 and Akt	Downregulation	[235]
miR-195	Blocks G1/S transition by repressing Rb/E2F signaling	Downregulation	[236]
miR-199a/b-3p	Suppress HCC growth through targeting PAK4 and inhibiting PAK4/Raf/MEK/ERK pathway	Downregulation	[237]
miR-21	Suppresses PTEN, hSulf-1, PDCD4, and RECK and activates EMT via AKT and ERK pathways	Overexpression	[238, 239]
miR-214	Contributes to angiogenesis through activation of HDGF paracrine pathway	Downregulation	[240]
miR-216a/217	Activates the PI3K/Akt and TGF-β pathways by targeting PTEN and SMAD7	Overexpression	[241]
miR-221	Accelerates hepatocyte proliferation during liver regeneration	Overexpression	[242]
miR-222	Promotes metastasis through activating AKT signaling and targeting PPP2R2A	Overexpression	[243]
miR-224	Activates AKT signaling pathway by targeting PPP2R1B	Overexpression	[244]
miR-26a	Inhibits angiogenesis by down-Regulating VEGFA through PIK3C2α/Akt/HIF-1α Suppress HCC growth and metastasis through IL-6-Stat3 signaling	Downregulation	[222, 223]
miR-27a	Reverses drug resistance (MDR) by inhibiting the FZD7/β-catenin pathway	Downregulation	[245]
miR-375	Inhibits autophagy by reducingATG7 expression	Downregulation	[246]

(*continued overleaf*)

Tab. 3 (*Continued.*)

microRNA	*Mechanism*	*Alteration in HCC*	*Reference(s)*
miR-503	Blocks G1/S transition by repressing Rb/E2F signaling	Downregulation	[247]
miR-519d	Targets CDKN1A/p21, PTEN, AKT3, and TIMP2. Promotes cell proliferation, invasion, and impairs apoptosis	Overexpression	[248]
miR-520b	Contributes to escape from growth suppression by targeting MEKK2 and cyclin D1 through JNK and Rb	Downregulation	[249]
miR-612	Suppresses EMT through Akt2	Downregulation	[250]
miR-675	Increases proliferation and inhibits invasiveness by downregulating RB and Twist1	Overexpression	[251]
miR-7	Inhibits HCC cell growth and metastasis by targeting PI3K/AKT pathway	Downregulation	[252]

Tab. 4 Epigenetic deregulations in HCC.

	Alteration in HCC	*Reference(s)*
DNA methyltransferases		
DNMT1, DNMT3A, DNMT3B	Upregulated	[256, 263–266]
Histone deacetylases		
HDAC-1, HDAC-2, HDAC-3	Upregulated	[267, 268]
SIRT1	Upregulated	[269, 270]
SIRT2	Upregulated	[271]
SIRT3	Downregulated	[272]
SIRT6	Downregulated	[273, 274]
Histone methyltransferases		
EZH2	Upregulated	[275, 276]
SUV39H1	Upregulated	[277]
SMYD3	Upregulated	[278]
MMSET (NSD2)	Upregulated	[279]

infection-associated disease, and its etiology is quite well known. Late diagnosis and the paucity of efficient therapeutic interventions are the major reasons why this cancer remains one of the most deadly on a worldwide basis. During the past decade, many of the investigations on liver cancer have focused on molecular classification methods and the identification of novel therapeutic targets and targeted agents. High-throughput analyses of the genomic state and genome-wide expression analyses were the most frequently used approaches for molecular classification, the primary purpose of which is to reduce the heterogeneity of HCC in terms of therapeutic response and patient survival. These studies have allowed the discovery of different molecular subtypes of liver cancer by using so-called "gene

Tab. 5 Hypermethylated and hypomethylated genes in HCC.

Hypermethylated genes	*Description*	*Role in HCC*	*Reference(s)*
RASSF1A	Ras association domain family member 1	Ras signaling pathway	[280–285]
RASSF5 (NORE1B)	Ras association domain family member 5	Ras signaling pathway	[281]
DAB2IP	DAB2 (mitogen-responsive phosphoprotein) interacting protein	Ras GTPase-activating protein	[283–285]
PTEN	Phosphatase and tensin homolog	PI3K/Akt/mTOR pathway	[280, 286]
TP53	Tumor protein p53	Survival, cell death, proliferation, growth	[287]
RB1	Retinoblastoma 1	Cell cycle, proliferation, growth	[288]
CDKN2A (p16)	Cyclin-dependent kinase inhibitor 2A	Cell cycle, proliferation, growth	[283–285, 289–293]
CDKN2B (p15)	Cyclin-dependent kinase inhibitor 2B	Cell cycle, proliferation, growth	[290, 294]
CDKL2	Cyclin-dependent kinase-like 2	Cell cycle, proliferation, growth	[283–285]
DACH1	Dachshund family transcription factor 1	Proliferation, growth	[295]
BMP4	Bone morphogenetic protein 4	Growth, metabolism, angiogenesis	[283–285]
BMP6	Bone morphogenetic protein 6	Growth, metabolism, angiogenesis	[296]
SOCS1	Suppressor of cytokine signaling 1	Jak/Stat pathway	[176, 297, 298]
SOCS3	Suppressor of cytokine signaling 3	Jak/Stat pathway	[174]
SYK	Spleen tyrosine kinase	Immune response	[299]
MAT1A	Methionine adenosyltransferase I, alpha (liver-specific)	Metabolism	[300–302]
GLS2	Glutaminase 2 (liver, mitochondrial)	Metabolism	[303]
GSTP1	Glutathione S-transferase pi 1	Metabolism	[282, 304, 305]
NQO1	NAD(P)H dehydrogenase, quinone 1	Metabolism	[306]
COX-1, COX-2	Cyclooxygenases	Metabolism	[307]
NKX6-2	NK6 homeobox 2	Metabolism	[283–285]
CDH1	Cadherin 1, type 1, E-cadherin	Invasion, metastasis	[308]
SFRPs	Secreted frizzled-related proteins	Wnt/β-catenin pathway	[309–312]

(*continued overleaf*)

Tab. 5 (*Continued.*)

Hypermethylated genes	*Description*	*Role in HCC*	*Reference(s)*
DACT2	Disheveled-binding antagonist of beta-catenin 2	Wnt/β-catenin pathway	[313, 314]
PRDM2 (RIZ1)	PR domain containing 2, with ZNF domain	Epigenetic regulation	[315, 316]
PRDM5	PR domain containing 5	Epigenetic regulation	[317]
CHD5	Chromodomain helicase DNA-binding protein 5	Chromatin remodeling	[318]
DNM3	Dynamin 3	Microtubule dynamics, vesicular transport	[283–285]
Hypomethylated genes	*Description*	*Role in HCC*	*Reference(s)*
MET	Met proto-oncogene	Growth, invasion, metastasis	[319]
AKT3	v-Akt murine thymoma viral oncogene homolog 3	PI3K/Akt/mTOR pathway	[283–285]
CD147	Basigin (ok blood group)	Invasion, metastasis	[257]
VIM	Vimentin	Invasion, metastasis	[260]
TFF3	Frequent trefoil factor 3	Inflammation, immune response	[320]
CCL20	Chemokine (C-C motif) ligand 20	Inflammation, immune response	[283–285]
CD1B	T-cell surface glycoprotein CD1b	Inflammation, immune response	[283–285]
CD1E	T-cell surface glycoprotein CD1e	Inflammation, immune response	[283–285]
CD300E	Immune receptor expressed on myeloid cells 2	Inflammation, immune response	[283–285]
MNDA	Myeloid cell nuclear differentiation antigen	Inflammation, immune response	[283–285]
MAT2A	Methionine adenosyltransferase 2, alpha	Metabolism	[302, 321]
CYP11B1	Cytochrome P450, family 11, subfamily B, polypeptide 1	Drug metabolism	[283–285]
LINE-1	Long interspersed nuclear element 1	Proliferation	[322]

signatures." A gene signature is the expression pattern of a set of genes associated with a clinical subtype of a disease, and in this regard several gene signatures and associated molecular types of HCC have been described. The heterogeneity of liver cancer, in parallel with the multistep evolution of this cancer, is represented in Figure 2. During the progression of HCC, various cellular mechanisms and

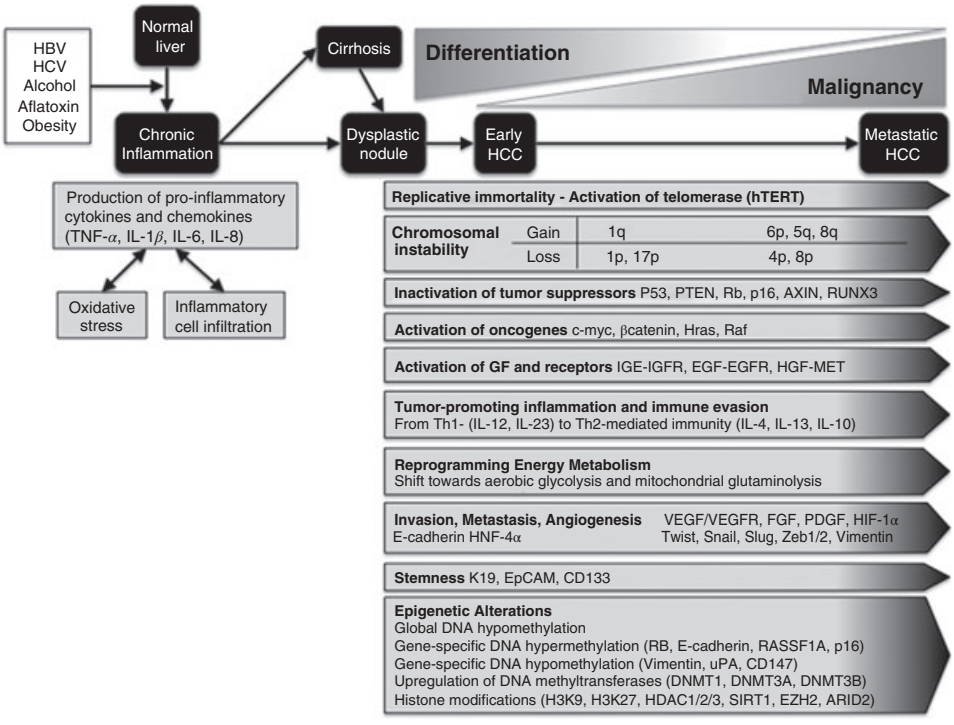

Fig. 2 Multistep evolution of primary liver cancer. The development of HCC is a multistep process, where injured hepatocytes promote chronic inflammation leading to hepatocyte death and regeneration cycles during cirrhosis and enduring liver disease. The subsequent expansion of dysplastic nodules, telomerase reactivation, increased genomic instability, inactivation of tumor suppressors, activation of oncogenes, and increase in growth factor signaling initiates HCC. Chromosomal instability and somatic mutations that favor the uncontrolled growth of HCC cells accumulate as the cancer advances. The acquisition of malignant phenotype is supported by tumor-promoting inflammation, a capability to evade immune destruction, and metabolic alterations that allow the continued growth and survival of cancer cells. Onset of invasive, metastatic, and angiogenetic capabilities promotes progression of carcinoma to the highly malignant metastatic state associated with stemness markers. Molecular alterations throughout the malignant transformation of HCC are regulated at both genetic and epigenetic levels.

their underlying genetic, epigenetic and proteomic alterations enable the acquisition of malignant behavior. These findings demonstrated that HCC is indeed a heterogeneous disease that can be subdivided into more or less homogeneous subclasses. Unfortunately, these findings were of very little assistance to the clinical follow-up of HCC patients, mainly because gene signatures are composed of large sets of genes and are not readily adaptable to routine use.

To date, the outcome of efforts to identify novel targets for HCC treatment has been less than satisfactory, and there are several reasons for this. First, the number of mutant but targetable genes is limited. Second, the pathogenesis of HCC may be related to cellular signaling pathways rather than to specific genes. It is probable that some of

the HCC-promoting signaling pathways are activated by mechanisms other than gene mutations. Changes in gene expression or protein networks (such as overall expression, stability, post-translational modifications) may be more critical for HCC pathology than are gene mutations. In HCC, the number of genes with dysregulated expression is extremely high, and consequently a large set of signaling pathways appear to be deregulated in this cancer. The paucity of gene mutations, together with such a high number of genes with expression changes, strongly suggests that the epigenome of HCC is highly affected. DNA methylation changes appear to predominate the HCC epigenome, while a few reported data on histone methylation patterns also indicate that profound changes occur in the organization of HCC nucleosomes and chromatin. Future studies aimed at deciphering the status of HCC epigenome and its effect on HCC proteome may lead to a better understanding of this unusual cancer, and lead in turn to the discovery of novel therapeutic targets.

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