Signaling Pathways as Potential Therapeutic Targets in Hepatocarcinogenesis

Article *in* Journal of Gastrointestinal Cancer · August 2017

DOI: 10.1007/s12029-017-9958-1

CITATIONS
0

READS
79

4 authors:

Yeliz Yılmaz
Dokuz Eylul University
6 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Aysim Gunes
Dokuz Eylul University
10 PUBLICATIONS **94** CITATIONS
SEE PROFILE

Hande Topel
Dokuz Eylul University
13 PUBLICATIONS **55** CITATIONS
SEE PROFILE

Neşe Atabey
Dokuz Eylul University
81 PUBLICATIONS **985** CITATIONS
SEE PROFILE

J Gastrointest Canc
DOI 10.1007/s12029-017-9958-1

REVIEW ARTICLE

Signaling Pathways as Potential Therapeutic Targets in Hepatocarcinogenesis

Yeliz Yılmaz[1,2] · Ayşim Güneş[1] · Hande Topel[1,2] · Neşe Atabey[1,3]

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and has a poor prognosis. HCC is described as a process with a complex molecular pathogenesis. There are three common mechanisms in the initiation of HCC: (i) liver injury, which is induced by etiologic factors such as chronic viral infections with hepatitis B and hepatitis C viruses, alcohol consumption, and obesity; (ii) fibrosis and cirrhosis, which are triggered after recurrent damage and regeneration cycles; and (iii) de-regulation of one or more oncogene and/or tumor suppressor gene [1–5].

Surgical resection, liver transplantation, radiofrequency ablation, and trans-catheter arterial chemoembolization (TACE) are the primary treatment methods for HCC patients. However, only 20% of patients are diagnosed at early stage of HCC and curative treatment options provide low survival rate estimated less than 5 years. In addition, hypervascularization, inflammation, fibrosis, and cirrhosis, which are hallmarks of HCC progression, complicate the early diagnosis of HCC. As a result, most of the patients are diagnosed at intermediate or advanced disease stages and curative treatments can only be applied to 30% of newly diagnosed patients [6]. In advanced HCC, median survival rate is extremely poor, 6 to 10 months. Sorafenib has been the only approved" drug for patients with advanced stage hepatocellular carcinoma until lately. Regorafenib has completed phase III trials and was found to increase overall survival of advanced stage HCC patients; it is granted priority review by Food and Drug Administration (FDA) early in this year for use in second-line systemic treatment. However, median overall survival of these HCC patients is still less than 1 year and further targeted therapies are needed urgently for advanced stage HCC patients [7, 8].

Growth factor signaling pathways taking part in every step of carcinogenesis are known to be deteriorated in cancer and specifically targeting elements of these pathways would increase overall survival with limited adverse effects. Besides, signaling pathways controlling hallmarks of cancer have great importance and should be acknowledged. In this review, we have focused on the most highlighted signaling pathways including receptor tyrosine kinases taking part in development and progression of HCC, stress-induced signaling like JAK-Stat or p53-Rb, pathways regulating stem cell fate like Notch-Hedgehog, signaling molecules that contribute to drug resistance, and the importance of receptor crosstalk in HCC (Fig. 1. However, other pathways controlling inflammation or metabolic reprogramming not mentioned here should also be appreciated (Fig. 1).

Signaling Pathways Implicated in HCC

Most cases of the HCC develop in the background of fibrotic/cirrhotic liver. The common outcome for hepatocarcinogenesis is chronic inflammation that is induced by secretion of pro-inflammatory cytokines from Kupffer cells, such as tumor necrosis factor α (TNFα), interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-1β (IL-1β). Following that parenchymal cells

✉ Neşe Atabey
nese.atabey@deu.edu.tr

[1] Izmir International Biomedicine & Genome Institute (iBG-izmir), Dokuz Eylul University, Balcova, 35340 Izmir, Turkey

[2] Department of Medical Biology and Genetics, Institute of Health Sciences, Dokuz Eylul University, 35340 Izmir, Turkey

[3] Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University, 35340 Izmir, Turkey

Published online: 18 August 2017

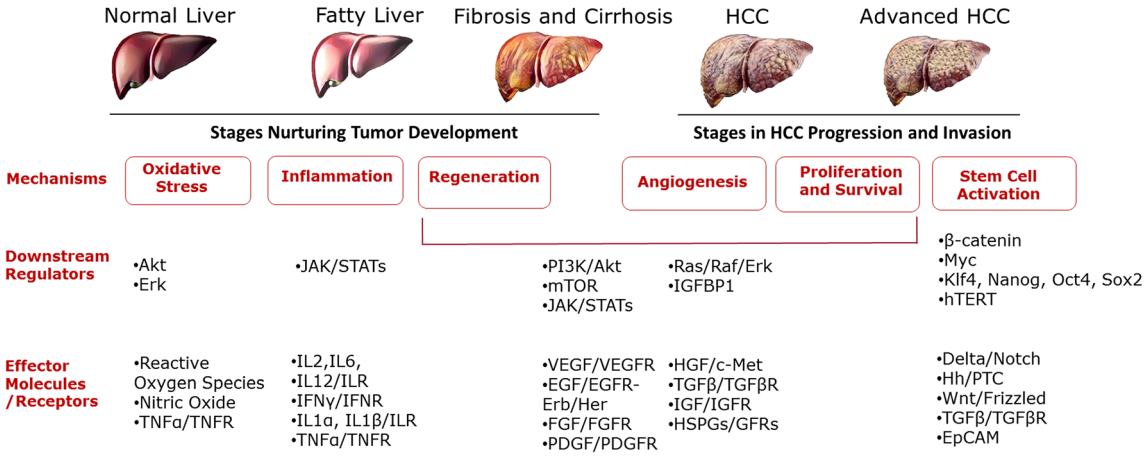

Fig. 1 Signaling pathways are activated in multiple stages of HCC development and progression. They activate inflammatory and oxidative stress-related molecules and promote regeneration in pre-cancerous liver. Proliferation and survival of tumor cells, angiogenesis, and stem cell activation contribute to cancer development and advanced stage HCC

are activated to promote regeneration process. If the liver injury persists, regeneration process is deregulated and liver architecture is changed via excessive deposition of extracellular matrix (ECM) components such as collagen 1. This process is determined as fibrosis and it is generated from wound healing response of injured liver. Biological responses such as cell proliferation, growth, and angiogenesis have central roles in that process [2].

Signaling Pathways in the Development of HCC

Several factors take role in the development of HCC. Chronic inflammation is inherent to constant hepatic injury, excessive regenerative process, and elevated oxidative stress resulting from hepatitis C infection or a background of non-alcoholic steatohepatitis (NASH) gives rise to the well-categorized stages of disease development, fibrosis, cirrhosis, and HCC [9–11]. Therefore, a deeper understanding of inflammatory signaling that controls oxidative stress, cell proliferation, survival, and differentiation, as well as crosstalk of these pathways, is vital to diagnose and treatment in HCC. In the scope of this review, we emphasize signaling elementary to hepatocarcinogenesis (Fig. 2).

Previous studies have shown that signaling pathways including growth factors and their relevant receptors have been altered in early hepatocarcinogenesis. Growth factors exert their multiple and overlapping functions in a paracrine/autocrine manner via binding to transmembranous receptor tyrosine kinases (RTKs). Ligand binding results in receptor dimerization and transphosphorylation of tyrosine residues by partner receptor. The phosphorylation of specific tyrosine residues creates binding sites for Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains on receptor. In the cytoplasm, specific proteins possessing these domains, lead to the initiation of signaling cascade. Other proteins that interact with the activated receptor act as adaptor proteins which link RTKs with their downstream effector proteins.

The most extensively determined proliferation, growth, and angiogenesis factors are vascular endothelial (VEGF), epidermal (EGF), fibroblast (FGF), platelet-derived (PDGF), and hepatocyte (HGF) growth factors in hepatocarcinogenesis [12]. Activated EGF receptor (EGFR) signaling in early stages of hepatocarcinogenesis has been reported. Overexpression of EGFR is observed 52–71% of the patients with HCC [13]. EGFR, which is a transmembrane protein, is also known as ErbB1/HER1. ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4 are other members of the EGFR family. The molecular mechanisms resulting from EGFR activation are receptor and/or ligand overexpression through autocrine and paracrine activation loops. In autocrine loop, the binding of EGF can homo or heterodimerize the EGFR that results in transphosphorylation. Transforming growth factor (TGF), amphiregulin (AR), heparin binding EGF (HB-EGF), betacellulin, and epiregulin are also defined as paracrine ligands of EGFR signaling. In the downstream of activated EGFR, there are several major signaling pathways such as Ras-ERK, p38 MAPK, PI3K/Akt, mTOR, and STAT [14, 15]. EGFR system plays a central hepatoprotective and regenerative role in the liver. Activated EGFR is also determined as an inducer of pro-inflammatory cytokine secretion. In stellate and liver myofibroflast cells, activated EGFR signaling regulates the expression levels of collagen, TGF, and connective tissue growth factor (CTGF) to contribute to the phenotypic activation of fibrosis [16, 17].

PDGF is also activated in an early stage of chronic inflammation. PDGF family members such as PDGF A, B, C, and D differently bind to two PDGF receptor (PDGFR-α and PDGFR-β) isoforms to activate downstream signaling cascade. PDGFR signaling is essential for the development of liver fibrosis in chronic hepatitis [2].

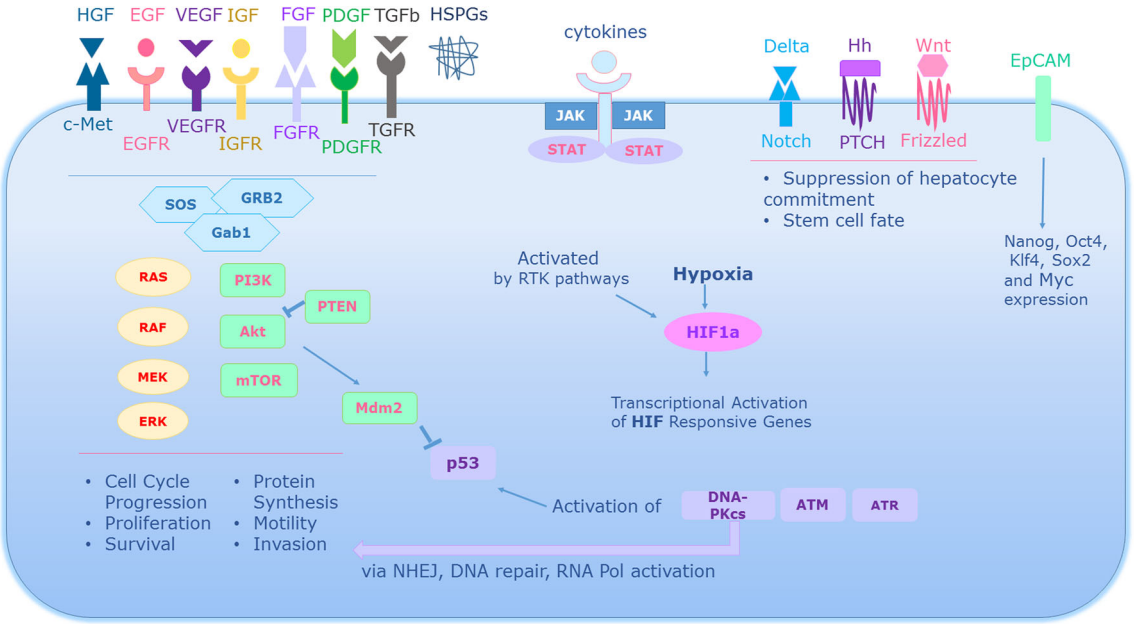

Fig. 2 Several pathways are activated during the development of HCC. Signal is transduced via downstream mediators which are shared among most of the signaling pathways to promote cell proliferation, survival, motility, invasion, and hence, metastasis

Liver regeneration also depends on effective angiogenesis. Angiogenesis is described as an autocrine and paracrine interactions between tumor cells, vascular endothelial cells, and pericytes. Besides, the existing microvasculature in the liver is destabilized, and to lead the vascular hyperpermeability, ECM is remodeled and endothelial cells are activated. The activated endothelial cells proliferate, migrate, and undergo cord formation to form new microvessels. Finally, pericytes are activated and the new blood vessels are stabilized [18]. Pro-angiogenic and anti-angiogenic factors are in a balance in normal angiogenesis process. In contrast, in the development and progression of HCC, angiogenic balance is disturbed and tumor, endothelial, and pericyte cells express angiogenic factors persistently. Sinusoidal capillarization, which is another neo-angiogenic process, has also been defined in early hepatocarcinogenesis. Transformation of hepatic sinusoids into continuous capillaries is the characteristic behavior of sinusoidal capillarization. Following that, collagenization of the extravascular spaces and deposition of laminin and basement membranes take place near the endothelial cells and hepatocytes [19]. Several studies have shown that VEGF-A, angiopoetin-2, FGF, PDGF, EGF, HGF, and IL-4, IL-6, IL-8 are the most common angiogenic growth factors in HCC development.

Related with that, VEGF is one of the pathways that is activated in liver regeneration. VEGF is transcribed, alternatively spliced, and translated into four different isoforms. Their functional effects are mediated through two types of receptor tyrosine kinases: VEGFR-1 and VEGFR-2 [20]. Although VEGF is determined as a hypoxia sensitive growth factor, hepatitis B virus infection and EGFR signaling upregulate VEGF expression and secretion in cirrhotic and dysplastic liver tissue. Raskopf and colleagues have determined that inhibition of VEGF production via small interfering RNA reduces endothelial cell proliferation, tube formation, and also tumor growth in HCC models [21].

FGF acts as a collaborator with VEGF signaling to promote pro-angiogenic signal and both of them are found to be overexpressed in HCC [22]. FGF receptor family includes four transmembrane receptors with tyrosine kinase activity (FGF 1-4), one receptor lacking cytoplasmic tyrosine kinase domain (FGFR-like1) [23]. FGF family consists of more than 20 members acting as ligands in signaling or hormones [24, 25]. FGF1, FGF2, and FGF19 are liver specific types of FGFs and FGFR1-2 and 4 are liver specific RTKs. These molecules are only detected in the liver tissues of patients with chronic hepatitis (CH) type C and HCC, but not in normal liver tissue [26]. The constitutively active form of FGFR has been involved in aberrant vascular formation. In addition to angiogenesis, FGFR exerts its functional role through cell proliferation and growth-related multiple downstream pathways such as Ras-ERK and PI3K/Akt. FGF-mediated Ras-ERK activation mainly regulates cellular proliferation and wound healing, while FGF-trigged PI3K-Akt activation provides cellular survival [27].

Ras-ERK is one of the pathways that are activated by growth factor signaling pathways in hepatocarcinogenesis. Ras proteins have intrinsic GTPase activity and function as a GDP/GTP-regulated switch. The GTP-bound form of Ras is characterized as an active and the GDP-bound form is characterized as inactive state. Guanine nucleotide exchange factors (GEFs) catalyze the replacement of GDP with GTP whereas

GTPase-activating proteins (GAPs) induce GTP hydrolysis to GDP. When Ras is activated, it binds to a downstream molecular target, Raf, and regulates cell growth, survival, differentiation, and migration [28]. Several human and animal studies have shown increased expression and activation of the Ras-ERK pathway is observed in hepatocarcinogenesis, compared to surrounding non-neoplastic liver tissue. Activated Raf-1 phosphorylates the MAP2Ks, MEK 1, and MEK 2, which in turn phosphorylate and activate ERK 1/2 [29–31]. ERK1/2 are transcription factors that, activate several other target molecules via binding to their promoters critical in cell proliferation and growth. PI3K and mTOR are also other proliferation and growth related signaling pathways, which are activated by Ras-ERK axis [32]. Ras also has critical roles in cell cycle regulation. For instance, Ras induces the expression of cyclin D1, represses $p27^{KIP1}$, and activates CDK4–cyclin D and CDK2–cyclin E complexes [33]. Ras-ERK also promotes cellular survival by repressing the expression or activity of pro-apoptotic molecules such as BCL-2 family members and Fas, Trail, and TNF [34].

Signaling Pathways in the Progression of HCC

Pathways related to HCC development and progression form a great majority of targets in cancer therapy and they control essential hallmarks of cancer such as sustained cell proliferation, resistance against apoptosis, genomic instability and escape from replicative senescence, induced angiogenesis and increased motility, invasion, and metastasis [35].

Due to the rapid proliferation of HCC cells, the tumor cells quickly exhaust nutrient and oxygen from the vasculature. Besides chronic liver injury, fibrogenesis also demolishes the liver blood system [36]. As a result, HCC cells become hypoxic. However, hypoxia is toxic to HCC cells and most of the cells are eliminated by cell death mechanisms, hypoxia also gives opportunity to the rest of the cells to induce adaptive "pro-survival" changes. Within that period, hypoxia-inducible factor 1 (HIF) is activated under hypoxic conditions. Ras-ERK, PI3K/Akt, and all other growth factor signaling pathways lead to the accumulation of HIF1α in both normoxic and hypoxic conditions [37, 38]. HIFs are transcription factors that are composed of an oxygen sensitive α subunit and constitutively expressed β subunit. Under hypoxic conditions, hydroxylation decreases due to inactivation of proline hydroxylases, leading to the inability of VHL to bind to HIF-1α and diminishes the degradation of HIF-1α. Stabilized HIF-1α, in turn, accumulates and translocates from the cytoplasm into the nucleus, where it dimerizes with HIF-1β and interacts with co-factors, such as p300/CBP, to bind to DNA on hypoxia response elements (HREs), ultimately activating target gene transcription and mRNA, and eventually protein synthesis [39].

HIF1α targets the genes which are essential for several biological processes that modulate tumorigenesis such as angiogenesis, glucose metabolism, survival, invasion, and metastasis. In this way, HIF1α promotes a metabolic and phenotypic switch that results in an adaptive stress response [40]. While cell cycle progress and proliferation are inhibited by HIF1α activation, HCC cells undergo "epithelial-mesenchymal transition (EMT)" in that period. EMT is a transient and reversible switch from polarized epithelial cells to mesenchymal cell phenotype. Following that, EMT exhibits highly motile, invasive, and aggressive cell phenotype [41]. Firstly, the upregulation of mesenchymal markers such as Snail, Twist, ZEB1, and ZEB2 and downregulation of epithelial markers such as E-cadherin are induced to change phenotypic properties of HCC cells [42]. Secondly, focal adhesions and stress fibers, which are critical for cell motility, comprise in the malign liver tissue. It has been determined that focal adhesion kinase (FAK), RhoA and Rac expressions, and activations are induced by HIF1α activation [43]. Ras-ERK, EGFR, FGF, and other growth factor signaling pathways also induce FAK, RhoA, and Rac expression and activation [44]. Following that, fillopodia-like cell morphology is developed and intracellular cytoskeleton architecture is changed such as formation of stress fibers. Thirdly, expression and secretion of proteases and matrix metalloproteinases including metalloproteinase-2 (MMP2) and MMP9, specific MMP types for liver, are induced to invade surrounding stroma and to intravasate the circulatory system. Finally, several researchers have determined VEGF, which is a target of HIF1α, is secreted from endothelial cells. VEGF is a specific mitogen and an inducer of angiogenesis. A higher level of VEGF expression in HCC is correlated with a higher proliferation index of endothelial cells, poor encapsulation of tumor tissue. Increased VEGF level is a predictive marker of disease outcome after curative treatments [45]. Increased levels of VEGF have been correlated with more invasive phenotype, shorter survival, and worse outcome [46]. Parallel to VEGF, VEGFR-1 and VEGFR-2 mRNA and protein expression are found to be high in HCC [47]. VEGF has also an important role in the disruption of tight junctions in HCC. In that way, VEGF enhances the invasion of HCC cells into normal liver parenchyma [48]. EGFR is also expressed highly in sinusoidal endothelial cells. A strong correlation is observed between betacellulin expression and EGFR activation, which is important for angiogenesis [49]. Platelet-derived endothelial cell growth factor (PD-ECGF) is another angiogenic factor that can promote endothelial cell proliferation and migration in HCC [50].

HGF/mesenchymal epithelial transition factor (Met) signaling is also one of the pathways, which is deregulated in hepatocarcinogenesis. In HCC, 80% of c-Met was found to be overexpressed when compared to surrounding non-tumoral tissue. Although c-Met is not active in normal liver tissue, it

is activated in early stages of hepatocarcinogenesis and this activation is highly correlated with liver regeneration, hepatocyte survival, and cell proliferation [51]. HGF is the most potent growth factor for hepatocyte cells. HGF binds to its high affinity receptor, c-Met, which is expressed dominantly in hepatocytes and endothelial cells. After chronic liver injury, c-Met is constitutively activated in liver tissue. The molecular mechanisms of constitutive c-Met activation are via autocrine and paracrine ligand production, genomic amplification, receptor crosstalk with other membrane receptors, and mutations in tyrosine residues of receptor. Activated c-Met signaling regulates multiple downstream effector molecules such as PI3K/Akt, Ras-ERK, and RhoA-Rac. Besides, activated c-Met signaling triggers the accumulation of HIF1α, VEGF, and glucose transporters (GLUTs) to promote EMT and to support tumor cells with oxygen and nutrients. c-Met activation also upregulates protease production and matrix metalloproteinase (MMPs) secretion and increased cell dissociation via extracellular matrix degradation, facilitating the motility and invasion of HCC cells [52–54].

One of the key mechanisms related to progression of cancer is a shift in cell metabolism. Reprogramming cell metabolism like in Warburg effect, which is known as a phenomenon of cancer cells metabolizing glucose in anaerobic glycolysis rather than oxidative pathways or a shift to oxidative phosphorylation (OXPHOS), is a fundamental of tumorigenesis [55]. Expression and activity of molecules controlling glucose metabolism and transport are altered in HCC. Among them, GLUT-1 and GLUT-2 are known to be upregulated and associated with worse prognosis in HCC patients, hexokinase family members, glyceraldehyde-3-phosphate (GAPDH), pyruvate kinases all take part in glycolysis and are also found to be upregulated in HCC progression, associated with worse prognosis and recurrence [56–60].

Stress-Mediated Signaling Pathways in HCC

Tumor cells respond and adapt to environmental signals such as oxidative stress, DNA damage, and inflammatory signals through multiple mechanisms that involve signal transduction processes. Since HCC usually develops in the context of hepatocyte injury and inflammation, ROS and nitrogen oxygen species generated by both tumor cells, inflammatory cells, and recurrent hypoxia-reoxygenation cycles induce hepatocarcinogenesis through several signaling pathways. In this part of the review, we will focus on some major signaling pathways regulating HCC progression.

The JAK/STAT Pathway The JAK (Janus Kinase)-STAT (Signal transducer and activator of transcription) signaling pathway is involved in transmitting information from extracellular polypeptide signals to target gene promoters. JAK/STAT pathway regulates innate and adaptive immune function, development, proliferation, differentiation, and apoptosis. Mammalian STAT proteins (STAT 1, 2, 3, 4, 5A, 5B, 6) contain tyrosine residues that are phosphorylated when the protein is active. STAT proteins may be dimerized either prior to tyrosine phosphorylation or after and both dimers are structurally different. STATs either have to form dimers in order to be phosphorylated (STAT4) or may be activated as monomers. Canonical STAT signaling is initiated by binding of a cytokine ligand to a cytokine receptor and subsequent activation of receptor-associated JAK family tyrosine kinases (JAK1, JAK2, JAK3, Tyk2) via transphosphorylation. Activation of JAKs promotes recruitment and activation of specific STATs. Activation of STAT pathway is not only initiated by cytokine receptors but also by some growth factor receptor tyrosine kinases, promoting STAT activation in a direct and indirect way. STAT proteins can both homo- and heterodimerize and dimers are imported to the nucleus where they promote transcription of various genes via cooperating with numerous transcription factors (IRFs, Sp1, Jun, Fos, NF-κB, GR) or chromatin remodeling coactivators (p300/CBP, PCAF, GCN5, BRG1, HDACs) [61].

Many genomic alterations of JAK/STAT pathway genes are reported to contribute to HCC, including mutations in JAK1 (9%), JAK2 (4.7%), IL6ST (3%), IL21R (3%), and PRL (3%); amplifications in IL6R (26%), IL20 (20%), and IL7 (19%); and deletions in PTPN6 (5%) [62, 63].

JAK-STAT3 pathway has been reported to have a crucial role in cancer inflammation and IL-6-JAK2-STAT3 signaling was found to accelerate incidence of HCC via promoting metabolic stress-induced inflammation in obese mice [64]. Activating mutations in gp130 signaling subunit of IL-6 contribute to HCC development when combined with β-catenin mutations. STAT3 can be activated via various cytokines but also by growth factors like EGF family and HGF in liver and it is reported to be activated in aggressive HCC tumor tissues. STAT3 can be activated in HCC cells by release of a panel of cytokines and growth factors including IL-6 by Kupffer cells, which is triggered by IL-1α induced NF-κB activation in a paracrine manner [65].

Some JAK/STAT pathway molecules such as STAT4 and STAT6 are reported to have tumor-suppressing or inhibiting effect in HCC. Related cytokines of STAT4 such as IL-12 (anti-tumoral effects in HCC via inducing IFNγ production), IFN-α (synergistically suppressing HCC growth with Sorafenib), and STAT4 itself (a SNP associated with lower levels of expression was reported as a risk factor in HCC) were shown to inhibit HCC growth [66]. Yang et al. reported that estrogen induced STAT6 activation represses HCC growth via inhibiting alternative activation of tumor associated macrophages (TAMs) [67].

DNA-PKcs Pathway DNA-PKcs is a member of phosphatidylinositol-3 (PI-3) kinase-like kinase family

(PIKK) and electrostatically binds to Ku-DNA complex following the recognition of double strand DNA ends by Ku-70/80 heterodimer to initiate non-homologous end joining (NHEJ) repair. In addition to its role in NHEJ, DNA-PKcs also contribute to BRCA1 mediated DNA repair, maintenance of telomere end-capping, immune system activation upon recognition of foreign DNA, RNA polymerase phosphorylation for transcriptional initiation, co-activation of androgen receptors, lipogenesis, mitosis, and regulation of Golgi apparatus [68–70].

DNA-PKcs is reported to be upregulated in liver cancer through four mechanisms: (1) amplification of the DNA-PKcs gene locus (8q11.21), (2) heat shock factor-1 (HSF1) induced and AP-1 mediated transcription of DNA-PKcs, (3) post-translational stabilization of DNA-PKcs protein by Reptin/RUBVL2, and (4) auto-phosphorylation and activation of DNA-PKcs mediated by physical interaction of TNKS1BP1, PARP1, and DNA-PKcs [70–72]. Altogether, these mechanisms contribute to DNA-PKcs upregulation in both HCC cell lines and tissues, which result in proliferation, survival, genomic instability, and microvessel density and lower survival rates of the patients [72, 73].

P53/Rb Pathway P53 maintains the integrity of the genome in response to cellular stress by means of initiating cell-cycle arrest, apoptosis, and senescence. Proteins derived from mutated p53 gene result in increased proliferation, survival, and metastatic capacity via taking the advantage of dominant-negative effect over the wild-type p53. P53 is primarily regulated by Murine Double Minute 2 (MDM2) protein which promotes poly-ubiquitination and proteasomal degradation of p53. DNA damage induces p53 phosphorylation, preventing Mdm2-p53 binding that leads to p53 stabilization and induced DNA damage response.

P53-mediated apoptosis is induced by pro-apoptotic Bax and Bak and the expression of those is reported to be reduced in HCC with mutated p53. Activation of wild-type p53 enhances the expression of insulin-like growth factor-binding protein-1 (IGFBP1) in HCC cells that antagonizes the mitochondrial apoptosis, so p53 activation promotes cell-cycle arrest rather than apoptosis [74]. p53 activation also induces the release of senescence-associated secretory factors to inhibit tumorigenesis by promoting a tumor-suppressive microenvironment in a paracrine manner. Ablation of p53-dependent senescence in hepatic stellate cells under chronic liver damage increases liver fibrosis and cirrhosis and also it enhances the transformation of adjacent epithelial cells into HCC [75–77].

Mutations of p53 contribute to HCC by decreasing the p53-mediated induction of Mdm2 and subsequently elevating the levels of mutated p53, lowering the affinity to bind the sequence-specific response elements. Unlike the other tissues, p53 is reported to be unique in terms of regulation. The expression of p53 seems to be much lower than the other tissues of the organism. In HCC, p53 mutations occur in aflatoxin-dependent and independent mechanisms, microdeletions of p14ARF (alternative reading frame product of CDKN2A locus), increased Mdm2 expression, and overexpression of gankyrin which inhibits both Retinoblastoma protein (Rb) and p53 checkpoint functions contribute to disruption of balance in Mdm2-p53 feedback and function [77–80]. A recent study showed that the pRb status regulates the induction of oxidative stress and mitochondrial production of ROS upon exposure to Sorafenib in HCC cells [81].

Telomere-, oncogene-, or ROS-dependent senescence stimuli induce different cyclin-dependent kinase inhibitors such as $p21^{Cip1}$, $p15^{INK4b}$, and $p16^{INK4a}$ to inhibit cyclin-dependent kinase mediated release of E2F factors from their inhibitory partner pRb. Inhibition of Rb phosphorylation by CDK4 and CDK2 promotes accumulation of senescent cells in G1 phase. P53 has a crucial role in regulation of DNA damage-induced senescence via inducing $p21^{Cip1}$-mediated CDK2 inhibition which prevents pRb phosphorylation. Chronic liver injury leading to hepatocarcinogenesis is through induced hepatocyte proliferation and progressive telomere-shortening. This is subsequently followed by bypassing of the senescence barrier of neoplastic cells by inactivating major senescence inducing genes like p53, $p15^{INK4b}$, and $p16^{INK4a}$. Acquiring the ability to proliferate by re-expressing hTERT enzyme, hepatocellular progression is provided via genomic instability and additional oncogenic alterations [35, 82].

TP53 is one of the most recurrently mutated genes in HCC and its mutation frequency average is approximately 26%. Alterations of the genes that belong to P53 signaling like ATM (upstream regulator of TP53), CDKN1A (target of TP53), and IRF2 (positive regulator of TP53) are also reported to be acquired in patients with HCC. Inactivation of RB and CDKN2A by homozygous deletion, point mutations, or promoter CpG hyper-methylation have also been reported in HCC patients to contribute to cell proliferation and genomic instability [63].

Stem Cell Fate

Cancer stem cells (CSCs) are very important properties of self-renewal, tumor initiation capacity, and tumor metastasis. Currently, liver CSCs are defined as an important targeting subset for the treatment of liver cancer. Surface antigens including CD133, CD90, CD44, EpCAM, and CD13 have been used to identify CSC properties in hepatocellular carcinoma. Stem cells of the liver are proposed to arise from two origins, either intrahepatic (hepatic progenitor cells (HPGs)) or extrahepatic (bone marrow and peripheral blood). In addition to major pathways that regulate stemness like Notch, Hedgehog, ant Wnt/β-catenin, EpCAM also has a crucial role in the maintenance of stem cell phenotype in CSCs. In this part, we reviewed current knowledge on some of the major signaling pathways that control stem cell fate in HCC.

The NOTCH Pathway The Notch signaling is a short-range communication transducer that is involved in proliferation, stem cell, and stem cell niche maintenance, cell-fate determination, differentiation, and also cell death during development and renewal of adult tissues. Notch signaling mediated by proteolysis and the amplitude and timing of Notch activity is regulated by post-translational modifications of ligands and receptors and also modulating their trafficking in a cellular context [83]. Four receptors (NOTCH 1–4), five canonical ligands (JAGGED1–2 and DELTA 1, 3, and 4), which generally act as activators, and some non-canonical ligands such as DLK1, DLK2/EGFL, EGFL7, and DNER (Delta/NOTCH-like epidermal growth factor (EGF)-related receptor) which act as inhibitors are expressed in mammalians [84, 85]. The release of Notch ligands from signal-sending cell is captured by Notch receptors of responding cell and through several cleavages, intracellular domain of Notch receptor becomes ready either to translocate to the nucleus where it acts as a transcriptional co-activator or interact with cytosolic proteins [86].

Notch signaling plays a crucial role in the differentiation of hepatoblasts during embryonic development giving rise to both hepatocytes and cholangiocytes [87]. Hepatocyte differentiation from hepatoblasts requires activation of HGF/MET and WNT pathways, whereas EGFR-induced NOTCH pathway activation is required for suppression of hepatocyte commitment and cholangiocyte differentiation [88, 89]. In adult liver, Notch signaling is activated in liver injury. Notch1 and Notch2 are expressed in hepatoblasts and cholangiocytes whereas Notch 3 and Notch 4 are expressed slightly in mesenchymal cells and endothelia following injury, also reported to be upregulated in cirrhosis [90]. Both loss and gain of function are reported to contribute to hepatoblastoma (HBT), hepatocellular carcinoma, and cholangiocarcinoma (CC). Studies on Notch1 receptor have not achieved a consensus whether Notch1 is a tumor suppressor or oncogene. While some studies reported that Notch1 overexpression results in inhibition of growth and proliferation, cell-cycle arrest, and apoptosis of HCC cells via interacting with Rb and p53 pathways, some others showed that Notch1 can promote cell proliferation and development of hepatocarcinogenesis and improve tumor size via collaborating with TNFα, NFkβ, RAS/MAPK, and PI3K/AKT/Hdm2 pathways and viral factors [91, 92]. Upregulated expression of Notch2 increases Cyclin D1 and Cyclin A2 levels and by interacting with AKT and NRAS pathways, it increases tumor burden of HCC. Role of Notch3 is more clearly defined in HCC and overexpression of Notch3 is correlated with aggressive phenotype, larger tumor bulk, and lower survival rates. Notch3 decreases the levels of p27, p53, and GADD45α; increases p21 expression; and promotes tumor progression by cooperating with EGFR/MAPK and PI3K/AKT pathways. In addition to Notch receptors, dysregulation of JAGGED1, DLL4, and DLK1 expression and activity contribute to liver cancer [91].

Some cancer studies show that Notch signaling prevents the expansion of premalignant stem/progenitor cells that carry oncogenic mutations by promoting their differentiation to the terminal stage. Upon loss of Notch signaling, cells get out of the control of Notch signaling which result in expansion of undifferentiated malignant cells, finally acquire secondary oncogenic hits to initiate tumor development. Loss of Notch signaling also promotes induction of pro-tumorigenic stroma that boosts tumor growth by maintaining an inflammatory niche. On the contrary of the opinion that Notch pathway suppress tumor formation and progression, Luo et al. showed that Notch pathway promotes the cancer stem cell characteristics of CD90+ HCC cells and inhibition of Notch pathway in CD90+ CSCs decreased tumorigenicity, cell invasion, and migration capacity [93].

The Hedgehog Pathway The Hedgehog (Hh) signaling pathway controls various processes during embryonic development and adult homeostasis. Developmental processes like tissue and organ patterning, proliferation, differentiation, stem cell maintenance, and maintaining bilateral asymmetry are controlled by Hedgehog signaling. Dysregulation of Hh signaling mechanism results in congenital defects and malformations. Mammalian Hh signaling comprise of three secreted ligands (Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH)), a protein that helps ligand release Dispatched, a negative regulatory receptor (Patched (PTCH), a positive regulatory protein located in the membrane (SMO), and the glioma-associated oncogene (GLI) transcription factors (GLI1, GLI2, and GLI3) that are translocated to the nucleus to activate expression of target genes. Hedgehog producing cell synthesizes Hedgehog protein through a ER/Golgi secretory pathway in which Hedgehog protein is processed by auto-proteolysis and Skinny Hedgehog (Ski/Skn) proteins that determine the range of its effect and stability also by tuning lipid modifications. Lipid modifications of the protein are important for the activity of the ligand and its release by Dispatched protein from the cell. Hh binds to trimeric receptor Patched (Ptc/Ptch1) by the participation of heparin sulfate proteoglycans (HSPGs) and additional co-receptors (Ihog family and Gas1). In the absence of Hh ligand, PTCH repress positive regulatory protein SMO activity by preventing its trafficking and localization on the cell membrane. Ligand binding to PTCH allows accumulation of SMO and its activation. Activated SMO modulates a signaling cascade that enables the activation by inhibiting the activity of its negative regulators (SUFU and GRK2) and release of GLI transcription factors from a protein complex that mediate its sequestration and proteasomal degradation. Translocation of GLI transcription factors result in expression of Hh target genes.

Hh signaling is largely inactive in the adult except the situations like tissue repair and maintenance in which Hh

activation is required for homeostasis. Activation of Hedgehog signaling also contributes to tumor formation, carcinogenesis, and metastasis via promoting proliferation, survival, migration, and invasion. Constitutive activation and dysregulation of Hh pathway in cancer is maintained in a ligand dependent and independent way. Ligand independent way occurs as a result of loss-of-function mutations of negative regulators (SUFU, PTCH) of the pathway or gain-of-function mutations of positive regulator SMO, GLI1, and GLI2. Epigenetic regulatory mechanisms also participate in ligand independent dysregulation. Ligand-dependent autocrine and paracrine activation of the pathway promotes tumor progression. Hh protein produced by cancer cells activates the own Hh pathway in an autocrine way, but also it activates the Hh pathway in normal stromal cells in a paracrine manner that results in the release of cytokines that promote survival and malignant phenotype of the cancer cell. Stromal cell Hh protein production and release also contribute to activation of Hh pathway in cancer cells [94, 95].

Abnormal activation of Hedgehog pathway contributing to hepatocellular carcinoma has been reported in the literature. Hedgehog signaling was reported to be activated in HCC cell lines, as well as in HCC tumor tissues. Upregulation of GLI1 mRNA and protein expression of HCC cell lines and HCC tissues were reported by various research groups. Expression of GLI1 is reported to be positively correlated with HCC recurrence after surgical resection and GLI1 expression found to induce epithelial to mesenchymal transition (EMT) via transcriptionally activating SNAI1 expression that is required for TGF-ß induced EMT process [96, 97]. GLI1 is reported to contribute to cell proliferation viability, migration, invasion, and colony formation of HCC cells by upregulating MMP-9 through ERK pathway [98]. GLI1 expression was reported as significantly upregulated in HCC tumor tissues compared to the tumor-adjacent normal tissues and GLI1 expression was positively correlated with intrahepatic metastasis, portal vein invasion, advanced TNM stage, and expression of SHH and Vimentin [99]. Sicklick et al. reported that whereas normal liver cells lack Hh pathway activity, HCC cells express Hh ligands Shh and Ihh, membrane protein Ptch1, proto-oncogene Smo, and transcription factor Gli1. They also reported that Smo expression is positively correlated with tumor size in HCC patients [100]. Tada et al. showed that Hedgehog interacting protein, a negative regulator of Hh signaling, is hypermethylated and transcriptionally downregulated in hepatoma cells which is an important evidence of epigenetic dysregulation of Hh signaling in HCC [101]. Chan et al. gave a metabolic insight to Hh research by defining the paracrine Hh ligand release from HCC cells inducing glycolytic metabolism of neighboring myofibroblasts which results in the release of myofibroblast-derived lactate, used as an energy source by HCC cells [102]. Activation of Hh pathway via upregulated expression of Hh ligands, regulating factors and effectors are reported to correlate with chronic infection of hepatitis B (HBV) and hepatitis C (HCV) viruses. Major cell populations that expand during HBV/HVC induced cirrhosis and HCC such as myofibroblasts, endothelial cells, and cancer stem cells were defined to be Hh responsive and promote liver fibrosis [103, 104]. In addition to viral infection induced liver fibrosis, Philips et al. reported that Hh pathway activation promotes liver fibrosis and hepatocarcinogenesis independently of viral infection and inhibition of Hh signaling reverses both processes even if they reach an advanced level [105]. In vivo xenograft studies clarified activation of Hh signaling in HCC and the mitigating effect of inhibition of Hh pathway [106].

The EpCAM Pathway Surface antigens including CD133, CD90, CD44, EpCAM, and CD13 have been used to identify cancer stem cell properties in hepatocellular carcinoma. Stem cells of the liver are proposed to arise from two origins, either intrahepatic (hepatic progenitor cells (HPGs)) or extrahepatic (bone marrow and peripheral blood). In addition to major pathways that regulate stemness like Notch, Hedgehog ant Wnt/β-catenin, EpCAM also has a crucial role in the maintenance of stem cell phenotype in cancer stem cells. EpCAM is a transmembrane protein that consists of an extracellular (EpEX), a single transmembrane and an intracellular (EpICD) domain. It is expressed during early liver development, in hepatic stem cells and hepatoblast whereas it is not expressed in hepatocytes. $EpCAM^+$ HCC cells express hepatic stem cell markers but not mature hepatocyte markers and have higher colony formation capacity than EpCAM (−) cells. As functioning as a cell-to-cell contact protein, EpCAM also transmits signals from membrane to nucleus to promote transcription of target genes. Sequential cleavage of EpCAM by TNFα converting enzyme (TACE/ADAM17) and a gamma-secretase complex containing presenilin 2 (PS-2) release its EpEX domain to the intercellular space and EpICD domain to cytoplasm. EpICD becomes a part of LEF/β-catenin transcription factor complex and induces transcription of various genes that take part in cell-cycle regulation and stemness. EpCAM regulates Nanog, Oct4, Klf4, Sox2, and Myc transcription in HCC cells [107]. Accumulation of β-catenin and activation of Tcf/β-catenin induce EpCAM expression [108]. Ji et al. reported inhibition of miR-181 reduces $EpCAM^+$ cell population and their ability to initiate tumor formation [109].

Even though they have not clarified the molecular mechanism, Su et al. pointed out a potential interaction between PTEN/AKT/mTOR pathway that HCC patients with $PTEN^-/CD133^+$ or $PTEN^-/EpCAM^+$ expression have a higher risk of recurrence and poor prognosis [110]. Karagonlar et al. demonstrated that adipocyte-derived cytokines induce motility and drug resistance in $EpCAM^+/CD133^+$ hepatic cancer stem cells through activating c-Met, Stat3, and ERK1/2 [111].

The Role of Signaling Molecules in Drug Resistance in HCC

In recent years, the development of anti-neoplastic drugs is a hot-topic research area. However, their limited curative efficacy, which is associated with multi-drug resistance (MDR), still remains unclear. The molecular mechanisms of drug resistance in cancer models are induction of EMT, HIF1α, and DNA damage repair, autophagy, epigenetic regulations, and receptor crosstalk.

Sorafenib, an oral multikinase inhibitor of Raf, VEGF, and PDGF molecules, is approved for advanced HCC [112]. One of the molecular mechanisms for Sorafenib resistance is the activation of other receptor tyrosine kinases via receptor crosstalk, by non-target receptors of sorafenib [113]. Firtina-Karagonlar and colleagues have described that c-Met is activated in sorafenib resistant cell clones and ERK1/2 are activated independently from Ras [114]. In furtherance, Xiang and colleagues propose a c-Met inhibitor (DE605) for combined therapy with Sorafenib in advanced HCC. In that study, it is shown that treatment of DE605 together with Sorafenib induces cellular apoptosis and inhibits tumor growth both in cell culture and animal models [115]. Besides, a randomized, placebo-controlled, and double-blind phase II study was reported for tivatinib, which is a selective oral inhibitor of c-Met. After 5 months, tivatinib group has a longer time to progress than placebo group [116].

Erlotinib is an orally active drug that inhibits EFGR tyrosine kinase activity. To evaluate the effect of erlotinib treatment combined with sorafenib, a randomized, placebo-controlled, and double-blind phase III is being studied. According to their preliminary results, erlotinib has no additional survival effect on advanced HCC patients [117].

New generation Bevacizumab is a VEGF inhibitor and it has been reported that median survival rate is 12.4 months in 46 HCC patients with no extrahepatic invasion [118]. Also, in early stages of hepatocarcinogenesis, the combination of bevacizumab with other cytotoxic agents, to normalize the tumor vasculature and to enhance the chemotherapy administration, presents current research area for researchers. The combination of bevacizumab with cytotoxic agents was evaluated in phase II study by several researchers. For example, Zhu and colleagues completed phase II study of the combination bevacizumab with oxaliplatin and gemcitabine [119].

The Role of Receptor Crosstalk in Hepatocarcinogenesis and Treatment Response in HCC

Lipid rafts play an important role in RTK crosstalk via bringing different receptors into proximity and thus promoting interactions between receptors and intracellular signaling proteins. Several researchers have determined four types of crosstalk: ligand, receptor, mediator, and downstream target molecule. When one of the RTKs are inhibited, to compensate the blockage of signaling, other types of RTKs are activated, which share the same downstream targets. Same mechanism is also obtained for ligands, mediators, and downstream effector molecules [120]. Jo and colleagues have described the cooperative action of c-Met and EGFR; this interaction facilitates c-Met activation in the absence of HGF [121]. EGF is also determined as a ligand for c-Met signaling. Following that, EGF is transcriptionally upregulated by c-Met activation-mediated Ras-ERK. Then, secreted EGF is transported out of the cell to activate EGFR in a paracrine way [122]. Besides, EGFR activation is also induced by c-Met activation through Ras-ERK signaling to activate metalloproteinase-3 (TIMP-3). TIMP-3 then cleaves c-Met from its ectodomain. The truncated c-Met promotes cell proliferation and differentiation [123]. In addition, cooperative action of c-Met and EGFR activate PI3K/Akt and Ras-ERK signaling in their downstream. EGFR can form complexes with PDGFR, insulin-like growth factor receptor (IGFR) to promote malign transformation of HCC cells.

In recent years, the interaction of RTKs with other membrane proteins has also been determined in previous studies. Korhan P and colleagues have shown that c-Met and Caveolin-1, which is a lipid raft membrane protein, co-localize in HCC cells and HGF induction enhances this interaction [124]. Also, same researchers have described that HGF-induced c-Met activation downregulates c-Met-Mucin 1 interaction in HCC cells [125].

Heparan sulfate proteoglycans (HSPGs) also regulate signaling pathways in hepatocarcinogenesis. Heparan sulfate (HS) comprise of variably sulphated and repeating disaccharide units. HS is synthesized in mast cells of the liver and with a post-translational modification; it is determined as HSPG. HSPGs are present on plasma membrane and in the ECM. They are important structural components of the basal membrane. Besides that, HSPGs constitute a protein core that have protein-binding domain to interact with various types of proteins. As a result, HSPGs have critical roles in signaling. HSPGs can bind to growth factors to facilitate or inhibit its binding to receptor. Besides, HSPGs might act as a low affinity receptor for growth factors to activate signaling pathway. Cell surface HSPGs act as receptors for adhesion molecules and growth factors and are involved in the regulation of cell proliferation, differentiation, motility, and invasion [126]. In HCC, glypican-3 (GPC-3) is determined as cell surface type of HSPGs. GPC-3 expression is significantly higher in HCC than that in normal liver tissues and non-tumorous liver tissues [127]. Suzuki M and colleagues determined that GPC-3 modulates signals such as Wnt, Hedgehog, and FGF. Kemp L and colleagues also showed that heparin enhances the stability of FGF-FGFR complex by crosslinking the ligand and receptor

[128, 129]. Heparin also binds to HGF and its receptor c-Met. In our previous studies, we have determined that heparin inhibits HGF induced cellular motility and invasion [130]. However, in the absence of HGF, heparin activated c-Met signaling and promoted motility and invasion in HCC cells. Heparin treatment led to c-Met receptor dimerization and activated c-Met signaling in an HGF-independent manner [131]. Receptor crosstalk is frequently associated with poor prognosis and resistance against traditional drug therapy, radiotherapy, and chemotherapies.

References

1. Guthle M, Dollinger MM. Epidemiology and risk factors of hepatocellular carcinoma. Radiologe. 2014;54(7):654–9. doi:10.1007/s00117-014-2650-6.
2. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. Nature Reviews Disease Primers. 2016;2:16018. doi:10.1038/nrdp.2016.18.
3. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterology. 2011;140(5):1410–26. doi:10.1053/j.gastro.2011.03.006.
4. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27. doi:10.1056/NEJMra1001683.
5. Fattovich G, Stroffolini T, Zagni I, Donato F (2004) Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 127 (5 Suppl 1):S35-50. doi:S0016508504015938.
6. Bruix J. Liver cancer: still a long way to go. Hepatology. 2011;54(1):1–2. doi:10.1002/hep.24468.
7. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma (2012). J Hepatol 56 (4):908–943. doi:10.1016/j.jhep.2011.12.001.
8. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;389(10064):56–66. doi:10.1016/S0140-6736(16)32453-9.
9. Bishayee A. The role of inflammation and liver cancer. Adv Exp Med Biol. 2014;816:401–35. doi:10.1007/978-3-0348-0837-8_16.
10. Takaki A, Yamamoto K. Control of oxidative stress in hepatocellular carcinoma: helpful or harmful? World J Hepatol. 2015;7(7):968–79. doi:10.4254/wjh.v7.i7.968.
11. Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol. 2014;14(3):181–94. doi:10.1038/nri3623 nri3623.
12. Zetter BR. Angiogenesis and tumor metastasis. Annu Rev Med. 1998;49:407–24. doi:10.1146/annurev.med.49.1.407.
13. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, et al. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol. 2005;23(27):6657–63. doi:10.1200/JCO.2005.14.696.
14. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol. 2006;7(7):505–16. doi:10.1038/nrm1962.
15. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. Epidermal growth factor receptor: mechanisms of activation and signalling. Exp Cell Res. 2003;284(1):31–53.
16. Perugorria MJ, Latasa MU, Nicou A, Cartagena-Lirola H, Castillo J, Goni S, et al. The epidermal growth factor receptor ligand amphiregulin participates in the development of mouse liver fibrosis. Hepatology. 2008;48(4):1251–61. doi:10.1002/hep.22437.
17. Berasain C, Perugorria MJ, Latasa MU, Castillo J, Goni S, Santamaria M, et al. The epidermal growth factor receptor: a link between inflammation and liver cancer. Exp Biol Med. 2009;234(7):713–25. doi:10.3181/0901-MR-12.
18. Papetti M, Herman IM. Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol. 2002;282(5):C947–70. doi:10.1152/ajpcell.00389.2001.
19. Kin M, Torimura T, Ueno T, Inuzuka S, Tanikawa K. Sinusoidal capillarization in small hepatocellular carcinoma. Pathol Int. 1994;44(10–11):771–8.
20. Ferrara N. The role of VEGF in the regulation of physiological and pathological angiogenesis. EXS. 2005;94:209–31.
21. Raskopf E, Vogt A, Sauerbruch T, Schmitz V. siRNA targeting VEGF inhibits hepatocellular carcinoma growth and tumor angiogenesis in vivo. J Hepatol. 2008;49(6):977–84. doi:10.1016/j.jhep.2008.07.022.
22. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, et al. Synergistic effect of basic fibroblast growth factor and vascular endothelial growth factor in murine hepatocellular carcinoma. Hepatology. 2002;35(4):834–42. doi:10.1053/jhep.2002.32541.
23. Ornitz DM, Itoh N. The fibroblast growth factor signaling pathway. Wiley Interdiscip Rev Dev Biol. 2015;4(3):215–66. doi:10.1002/wdev.176.
24. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer. 2010;10(2):116–29. doi:10.1038/nrc2780 nrc2780.
25. Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova AV, et al. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem. 2007;282(37):26687–95. doi:10.1074/jbc.M704165200.
26. Tsunematsu H, Tatsumi T, Kohga K, Yamamoto M, Aketa H, Miyagi T, et al. Fibroblast growth factor-2 enhances NK sensitivity of hepatocellular carcinoma cells. Int J Cancer. 2012;130(2):356–64. doi:10.1002/ijc.26003.
27. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov. 2009;8(3):235–53. doi:10.1038/nrd2792.
28. Rajalingam K, Schreck R, Rapp UR, Albert S. Ras oncogenes and their downstream targets. Biochim Biophys Acta. 2007;1773(8):1177–95. doi:10.1016/j.bbamcr.2007.01.012.
29. Ito Y, Sasaki Y, Horimoto M, Wada S, Tanaka Y, Kasahara A, et al. Activation of mitogen-activated protein kinases/extracellular signal-regulated kinases in human hepatocellular carcinoma. Hepatology. 1998;27(4):951–8. doi:10.1002/hep.510270409.
30. Schmidt CM, McKillop IH, Cahill PA, Sitzmann JV. Increased MAPK expression and activity in primary human hepatocellular carcinoma. Biochem Biophys Res Commun. 1997;236(1):54–8. doi:10.1006/bbrc.1997.6840.
31. McKillop IH, Schmidt CM, Cahill PA, Sitzmann JV. Altered expression of mitogen-activated protein kinases in a rat model of experimental hepatocellular carcinoma. Hepatology. 1997;26(6):1484–91. doi:10.1002/hep.510260615.
32. Chappell WH, Steelman LS, Long JM, Kempf RC, Abrams SL, Franklin RA, Basecke J, Stivala F, Donia M, Fagone P, Malaponte G, Mazzarino MC, Nicoletti F, Libra M, Maksimovic-Ivanic D, Mijatovic S, Montalto G, Cervello M, Laidler P, Milella M, Tafuri A, Bonati A, Evangelisti C, Cocco L, Martelli AM, McCubrey JA (2011) Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR inhibitors: rationale and importance to inhibiting these pathways in

human health. Oncotarget 2 (3):135-164. doi:10.18632/oncotarget.240.
33. Maurer G, Tarkowski B, Baccarini M. Raf kinases in cancer-roles and therapeutic opportunities. Oncogene. 2011;30(32):3477–88. doi:10.1038/onc.2011.160.
34. Ballif BA, Blenis J. Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. Cell Growth Differ. 2001;12(8):397–408.
35. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. doi:10.1016/j.cell.2011.02.013.
36. Wu XZ, Xie GR, Chen D. Hypoxia and hepatocellular carcinoma: the therapeutic target for hepatocellular carcinoma. J Gastroenterol Hepatol. 2007;22(8):1178–82. doi:10.1111/j.1440-1746.2007.04997.x.
37. Mazure NM, Chen EY, Yeh P, Laderoute KR, Giaccia AJ. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. Cancer Res. 1996;56(15):3436–40.
38. Sheta EA, Trout H, Gildea JJ, Harding MA, Theodorescu D. Cell density mediated pericellular hypoxia leads to induction of HIF-1alpha via nitric oxide and Ras/MAP kinase mediated signaling pathways. Oncogene. 2001;20(52):7624–34. doi:10.1038/sj.onc.1204972.
39. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science. 2001;294(5545):1337–40. doi:10.1126/science.1066373.
40. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 1998;12(2):149–62.
41. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7(2):131–42. doi:10.1038/nrm1835.
42. Zhang Q, Bai X, Chen W, Ma T, Hu Q, Liang C, et al. Wnt/beta-catenin signaling enhances hypoxia-induced epithelial-mesenchymal transition in hepatocellular carcinoma via crosstalk with hif-1alpha signaling. Carcinogenesis. 2013;34(5):962–73. doi:10.1093/carcin/bgt027.
43. Ye LY, Chen W, Bai XL, Xu XY, Zhang Q, Xia XF, et al. Hypoxia-induced epithelial-to-mesenchymal transition in hepatocellular carcinoma induces an immunosuppressive tumor microenvironment to promote metastasis. Cancer Res. 2016;76(4):818–30. doi:10.1158/0008-5472.CAN-15-0977.
44. Schlaepfer DD, Hunter T. Focal adhesion kinase overexpression enhances ras-dependent integrin signaling to ERK2/mitogen-activated protein kinase through interactions with and activation of c-Src. J Biol Chem. 1997;272(20):13189–95.
45. Poon RT, Lau C, Pang R, Ng KK, Yuen J, Fan ST. High serum vascular endothelial growth factor levels predict poor prognosis after radiofrequency ablation of hepatocellular carcinoma: importance of tumor biomarker in ablative therapies. Ann Surg Oncol. 2007;14(6):1835–45. doi:10.1245/s10434-007-9366-z.
46. Poon RT, Lau CP, Ho JW, Yu WC, Fan ST, Wong J. Tissue factor expression correlates with tumor angiogenesis and invasiveness in human hepatocellular carcinoma. Clin Cancer Res. 2003;9(14):5339–45.
47. Dhar DK, Naora H, Yamanoi A, Ono T, Kohno H, Otani H, et al. Requisite role of VEGF receptors in angiogenesis of hepatocellular carcinoma: a comparison with angiopoietin/tie pathway. Anticancer Res. 2002;22(1A):379–86.
48. Schmitt M, Horbach A, Kubitz R, Frilling A, Haussinger D. Disruption of hepatocellular tight junctions by vascular endothelial growth factor (VEGF): a novel mechanism for tumor invasion. J Hepatol. 2004;41(2):274–83. doi:10.1016/j.jhep.2004.04.035.
49. Ueda S, Basaki Y, Yoshie M, Ogawa K, Sakisaka S, Kuwano M, et al. PTEN/Akt signaling through epidermal growth factor receptor is prerequisite for angiogenesis by hepatocellular carcinoma cells that is susceptible to inhibition by gefitinib. Cancer Res. 2006;66(10):5346–53. doi:10.1158/0008-5472.CAN-05-3684.
50. Griffiths L, Stratford IJ. Platelet-derived endothelial cell growth factor thymidine phosphorylase in tumour growth and response to therapy. Br J Cancer. 1997;76(6):689–93.
51. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. Proc Natl Acad Sci USA. 2004;101(13):4477–82. doi:10.1073/pnas.0306068101.
52. Tavian D, De Petro G, Benetti A, Portolani N, Giulini SM, Barlati S. u-PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte growth factor mRNA is down-regulated in human hepatocellular carcinoma. Int J Cancer. 2000;87(5):644–9.
53. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res. 2013;19(9):2310–8. doi:10.1158/1078-0432.CCR-12-2791.
54. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. Nat Rev Cancer. 2012;12(2):89–103. doi:10.1038/nrc3205.
55. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv. 2016;2(5):e1600200. doi:10.1126/sciadv.1600200.
56. Amann T, Maegdefrau U, Hartmann A, Agaimy A, Marienhagen J, Weiss TS, et al. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. Am J Pathol. 2009;174(4):1544–52. doi:10.2353/ajpath.2009.080596.
57. Daskalow K, Pfander D, Weichert W, Rohwer N, Thelen A, Neuhaus P, et al. Distinct temporospatial expression patterns of glycolysis-related proteins in human hepatocellular carcinoma. Histochem Cell Biol. 2009;132(1):21–31. doi:10.1007/s00418-009-0590-4.
58. Gong L, Cui Z, Chen P, Han H, Peng J, Leng X. Reduced survival of patients with hepatocellular carcinoma expressing hexokinase II. Med Oncol. 2012;29(2):909–14. doi:10.1007/s12032-011-9841-z.
59. Chen Z, Lu X, Wang Z, Jin G, Wang Q, Chen D, Chen T, Li J, Fan J, Cong W, Gao Q, He X (2015) Co-expression of PKM2 and TRIM35 predicts survival and recurrence in hepatocellular carcinoma. Oncotarget 6 (4):2538-2548. doi: 10.18632/oncotarget.2991.
60. Shang RZ, Qu SB, Wang DS. Reprogramming of glucose metabolism in hepatocellular carcinoma: progress and prospects. World J Gastroenterol. 2016;22(45):9933–43. doi:10.3748/wjg.v22.i45.9933.
61. Mertens C, Darnell JE Jr. SnapShot: JAK-STAT signaling. Cell. 2007;131(3):612. doi:10.1016/j.cell.2007.10.033.
62. Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, et al. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res. 2013;23(9):1422–33. doi:10.1101/gr.154492.113.
63. Shibata T, Aburatani H. Exploration of liver cancer genomes. Nat Rev Gastroenterol Hepatol. 2014;11(6):340–9. doi:10.1038/nrgastro.2014.6.
64. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010;140(2):197–208. doi:10.1016/j.cell.2009.12.052.
65. He G, Karin M. NF-kappaB and STAT3—key players in liver inflammation and cancer. Cell Res. 2011;21(1):159–68. doi:10.1038/cr.2010.183.
66. Wang Y, Qu A, Wang H. Signal transducer and activator of transcription 4 in liver diseases. Int J Biol Sci. 2015;11(4):448–55. doi:10.7150/ijbs.11164.
67. Yang W, Lu Y, Xu Y, Xu L, Zheng W, Wu Y, et al. Estrogen represses hepatocellular carcinoma (HCC) growth via inhibiting alternative activation of tumor-associated macrophages (TAMs). J

Biol Chem. 2012;287(48):40140–9. doi:10.1074/jbc.M112.348763.
68. Farber-Katz SE, Dippold HC, Buschman MD, Peterman MC, Xing M, Noakes CJ, et al. DNA damage triggers Golgi dispersal via DNA-PK and GOLPH3. Cell. 2014;156(3):413–27. doi:10.1016/j.cell.2013.12.023.
69. Goodwin JF, Knudsen KE. Beyond DNA repair: DNA-PK function in cancer. Cancer Discov. 2014;4(10):1126–39. doi:10.1158/2159-8290.cd-14-0358.
70. Pascale RM, Joseph C, Latte G, Evert M, Feo F, Calvisi DF. DNA-PKcs: a promising therapeutic target in human hepatocellular carcinoma? DNA Repair (Amst). 2016;47:12–20. doi:10.1016/j.dnarep.2016.10.004.
71. Zou LH, Shang ZF, Tan W, Liu XD, Xu QZ, Song M, Wang Y, Guan H, Zhang SM, Yu L, Zhong CG, Zhou PK (2015) TNKS1BP1 functions in DNA double-strand break repair though facilitating DNA-PKcs autophosphorylation dependent on PARP-1. Oncotarget 6 (9):7011-7022. doi:10.18632/oncotarget.3137.
72. Evert M, Frau M, Tomasi ML, Latte G, Simile MM, Seddaiu MA, et al. Deregulation of DNA-dependent protein kinase catalytic subunit contributes to human hepatocarcinogenesis development and has a putative prognostic value. Br J Cancer. 2013;109(10):2654–64. doi:10.1038/bjc.2013.606.
73. Cornell L, Munck JM, Alsinet C, Villanueva A, Ogle L, Willoughby CE, et al. DNA-PK-A candidate driver of hepatocarcinogenesis and tissue biomarker that predicts response to treatment and survival. Clin Cancer Res. 2015;21(4):925–33. doi:10.1158/1078-0432.ccr-14-0842.
74. Leu JI, George DL. Hepatic IGFBP1 is a prosurvival factor that binds to BAK, protects the liver from apoptosis, and antagonizes the proapoptotic actions of p53 at mitochondria. Genes Dev. 2007;21(23):3095–109. doi:10.1101/gad.1567107.
75. Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE, et al. Non-cell-autonomous tumor suppression by p53. Cell. 2013;153(2):449–60. doi:10.1016/j.cell.2013.03.020.
76. Amaral JD, Castro RE, Steer CJ, Rodrigues CM. p53 and the regulation of hepatocyte apoptosis: implications for disease pathogenesis. Trends Mol Med. 2009;15(11):531–41. doi:10.1016/j.molmed.2009.09.005.
77. Meng X, Franklin DA, Dong J, Zhang Y. MDM2-p53 pathway in hepatocellular carcinoma. Cancer Res. 2014;74(24):7161–7. doi:10.1158/0008-5472.can-14-1446.
78. Brown CJ, Cheok CF, Verma CS, Lane DP. Reactivation of p53: from peptides to small molecules. Trends Pharmacol Sci. 2011;32(1):53–62. doi:10.1016/j.tips.2010.11.004.
79. Muller PA, Vousden KH. p53 mutations in cancer. Nat Cell Biol. 2013;15(1):2–8. doi:10.1038/ncb2641.
80. Pietsch EC, Sykes SM, McMahon SB, Murphy ME. The p53 family and programmed cell death. Oncogene. 2008;27(50):6507–21. doi:10.1038/onc.2008.315.
81. Louandre C, Marcq I, Bouhlal H, Lachaier E, Godin C, Saidak Z, Francois C, Chatelain D, Debuysscher V, Barbare JC, Chauffert B, Galmiche A (2015) The retinoblastoma (Rb) protein regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma cells. Cancer Lett 356 (2 Pt B):971-977. doi:10.1016/j.canlet.2014.11.014.
82. Ozturk M, Arslan-Ergul A, Bagislar S, Senturk S, Yuzugullu H. Senescence and immortality in hepatocellular carcinoma. Cancer Lett. 2009;286(1):103–13. doi:10.1016/j.canlet.2008.10.048.
83. Ilagan MX, Kopan R. SnapShot: notch signaling pathway. Cell. 2007;128(6):1246. doi:10.1016/j.cell.2007.03.011.
84. Katsube K, Sakamoto K. Notch in vertebrates—molecular aspects of the signal. Int J Dev Biol. 2005;49(2–3):369–74. doi:10.1387/ijdb.041950kk.
85. D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. Curr Top Dev Biol. 2010;92:73–129. doi:10.1016/s0070-2153(10)92003-6.
86. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009;137(2):216–33. doi:10.1016/j.cell.2009.03.045.
87. Tanimizu N, Miyajima A. Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. J Cell Sci. 2004;117(Pt 15):3165–74. doi:10.1242/jcs.01169.
88. Fiorotto R, Raizner A, Morell CM, Torsello B, Scirpo R, Fabris L, et al. Notch signaling regulates tubular morphogenesis during repair from biliary damage in mice. J Hepatol. 2013;59(1):124–30. doi:10.1016/j.jhep.2013.02.025.
89. Morell CM, Fiorotto R, Fabris L, Strazzabosco M. Notch signalling beyond liver development: emerging concepts in liver repair and oncogenesis. Clin Res Hepatol Gastroenterol. 2013;37(5):447–54. doi:10.1016/j.clinre.2013.05.008.
90. Gramantieri L, Giovannini C, Lanzi A, Chieco P, Ravaioli M, Venturi A, et al. Aberrant Notch3 and Notch4 expression in human hepatocellular carcinoma. Liver Int. 2007;27(7):997–1007. doi:10.1111/j.1478-3231.2007.01544.x.
91. Gil-Garcia B, Baladron V. The complex role of NOTCH receptors and their ligands in the development of hepatoblastoma, cholangiocarcinoma and hepatocellular carcinoma. Biol Cell. 2016;108(2):29–40. doi:10.1111/boc.201500029.
92. Villanueva A, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, et al. Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. Gastroenterology. 2012;143(6):1660–1669 e1667. doi:10.1053/j.gastro.2012.09.002.
93. Luo J, Wang P, Wang R, Wang J, Liu M, Xiong S, Li Y, Cheng B (2016) The Notch pathway promotes the cancer stem cell characteristics of CD90+ cells in hepatocellular carcinoma. Oncotarget 7 (8):9525-9537. doi:10.18632/oncotarget.6672.
94. Amakye D, Jagani Z, Dorsch M. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. Nat Med. 2013;19(11):1410–22. doi:10.1038/nm.3389.
95. Chen MH, Wilson CW, Chuang PT. SnapShot: hedgehog signaling pathway. Cell. 2007;130(2):386. doi:10.1016/j.cell.2007.07.017.
96. Zheng X, Vittar NB, Gai X, Fernandez-Barrena MG, Moser CD, Hu C, et al. The transcription factor GLI1 mediates TGFbeta1 driven EMT in hepatocellular carcinoma via a SNAI1-dependent mechanism. PLoS One. 2012;7(11):e49581. doi:10.1371/journal.pone.0049581.
97. Wang Y, Han C, Lu L, Magliato S, Wu T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. Hepatology. 2013;58(3):995–1010. doi:10.1002/hep.26394.
98. Lu JT, Zhao WD, He W, Wei W. Hedgehog signaling pathway mediates invasion and metastasis of hepatocellular carcinoma via ERK pathway. Acta Pharmacol Sin. 2012;33(5):691–700. doi:10.1038/aps.2012.24.
99. Xu QR, Zheng X, Zan XF, Yao YM, Yang W, Liu QG (2012) [Gli1 expression and its relationship with the expression of Shh, Vimentin and E-cadherin in human hepatocellular carcinoma]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 28 (5):536–539.
100. Sicklick JK, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, et al. Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis. 2006;27(4):748–57. doi:10.1093/carcin/bgi292.
101. Tada M, Kanai F, Tanaka Y, Tateishi K, Ohta M, Asaoka Y, et al. Down-regulation of hedgehog-interacting protein through genetic and epigenetic alterations in human hepatocellular carcinoma. Clin Cancer Res. 2008;14(12):3768–76. doi:10.1158/1078-0432.ccr-07-1181.

102. Chan IS, Guy CD, Chen Y, Lu J, Swiderska-Syn M, Michelotti GA, et al. Paracrine Hedgehog signaling drives metabolic changes in hepatocellular carcinoma. Cancer Res. 2012;72(24):6344–50. doi:10.1158/0008-5472.can-12-1068.
103. Arzumanyan A, Sambandam V, Clayton MM, Choi SS, Xie G, Diehl AM, et al. Hedgehog signaling blockade delays hepatocarcinogenesis induced by hepatitis B virus X protein. Cancer Res. 2012;72(22):5912–20. doi:10.1158/0008-5472.can-12-2329.
104. Pereira Tde A, Witek RP, Syn WK, Choi SS, Bradrick S, Karaca GF, et al. Viral factors induce Hedgehog pathway activation in humans with viral hepatitis, cirrhosis, and hepatocellular carcinoma. Lab Investig. 2010;90(12):1690–703. doi:10.1038/labinvest.2010.147.
105. Philips GM, Chan IS, Swiderska M, Schroder VT, Guy C, Karaca GF, et al. Hedgehog signaling antagonist promotes regression of both liver fibrosis and hepatocellular carcinoma in a murine model of primary liver cancer. PLoS One. 2011;6(9):e23943. doi:10.1371/journal.pone.0023943.
106. Jeng KS, Jeng CJ, Jeng WJ, Sheen IS, Chang CF, Hsiau HI, et al. Sonic hedgehog pathway inhibitor mitigates mouse hepatocellular carcinoma. Am J Surg. 2015;210(3):554–60. doi:10.1016/j.amjsurg.2015.03.001.
107. Oishi N, Yamashita T, Kaneko S. Molecular biology of liver cancer stem cells. Liver Cancer. 2014;3(2):71–84. doi:10.1159/000343863.
108. Yamashita T, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. Cancer Res. 2007;67(22):10831–9. doi:10.1158/0008-5472.CAN-07-0908.
109. Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. Hepatology. 2009;50(2):472–80. doi:10.1002/hep.22989.
110. Su R, Nan H, Guo H, Ruan Z, Jiang L, Song Y, et al. Associations of components of PTEN/AKT/mTOR pathway with cancer stem cell markers and prognostic value of these biomarkers in hepatocellular carcinoma. Hepatol Res. 2016;46(13):1380–91. doi:10.1111/hepr.12687.
111. Firtina Karagonlar Z, Koc D, Sahin E, Avci ST, Yilmaz M, Atabey N, et al. Effect of adipocyte-secreted factors on EpCAM+/CD133+ hepatic stem cell population. Biochem Biophys Res Commun. 2016;474(3):482–90. doi:10.1016/j.bbrc.2016.04.137.
112. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, et al. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Cancer Res. 2006;66(24):11851–8. doi:10.1158/0008-5472.CAN-06-1377.
113. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al., Group SIS. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90. doi:10.1056/NEJMoa0708857.
114. Firtina Karagonlar Z, Koc D, Iscan E, Erdal E, Atabey N. Elevated hepatocyte growth factor expression as an autocrine c-Met activation mechanism in acquired resistance to sorafenib in hepatocellular carcinoma cells. Cancer Sci. 2016;107(4):407–16. doi:10.1111/cas.12891.
115. Jiang X, Feng K, Zhang Y, Li Z, Zhou F, Dou H, Wang T (2015) Sorafenib and DE605, a novel c-Met inhibitor, synergistically suppress hepatocellular carcinoma. Oncotarget 6 (14):12340-12356. doi: 10.18632/oncotarget.3656.
116. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol. 2013;14(1):55–63. doi:10.1016/S1470-2045(12)70490-4.
117. Finn RS. Emerging targeted strategies in advanced hepatocellular carcinoma. Semin Liver Dis. 2013;33(Suppl 1):S11–9. doi:10.1055/s-0033-1333632.
118. Siegel AB, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol. 2008;26(18):2992–8. doi:10.1200/JCO.2007.15.9947.
119. Zhu AX, Blaszkowsky LS, Ryan DP, Clark JW, Muzikansky A, Horgan K, et al. Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24(12):1898–903. doi:10.1200/JCO.2005.04.9130.
120. Yamaguchi H, Chang SS, Hsu JL, Hung MC. Signaling cross-talk in the resistance to HER family receptor targeted therapy. Oncogene. 2014;33(9):1073–81. doi:10.1038/onc.2013.74.
121. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem. 2000;275(12):8806–11.
122. Reznik TE, Sang Y, Ma Y, Abounader R, Rosen EM, Xia S, et al. Transcription-dependent epidermal growth factor receptor activation by hepatocyte growth factor. Mol Cancer Res. 2008;6(1):139–50. doi:10.1158/1541-7786.MCR-07-0236.
123. Nath D, Williamson NJ, Jarvis R, Murphy G. Shedding of c-Met is regulated by crosstalk between a G-protein coupled receptor and the EGF receptor and is mediated by a TIMP-3 sensitive metalloproteinase. J Cell Sci. 2001;114(Pt 6):1213–20.
124. Korhan P, Erdal E, Kandemis E, Cokakli M, Nart D, Yilmaz F, et al. Reciprocal activating crosstalk between c-Met and caveolin 1 promotes invasive phenotype in hepatocellular carcinoma. PLoS One. 2014;9(8):e105278. doi:10.1371/journal.pone.0105278.
125. Bozkaya G, Korhan P, Cokakli M, Erdal E, Sagol O, Karademir S, et al. Cooperative interaction of MUC1 with the HGF/c-Met pathway during hepatocarcinogenesis. Mol Cancer. 2012;11:64. doi:10.1186/1476-4598-11-64.
126. Vlodavsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. Cancer Metastasis rev. 1990;9(3):203–26.
127. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology. 2003;125(1):89–97.
128. Suzuki M, Sugimoto K, Tanaka J, Tameda M, Inagaki Y, Kusagawa S, et al. Up-regulation of glypican-3 in human hepatocellular carcinoma. Anticancer Res. 2010;30(12):5055–61.
129. Kemp LE, Mulloy B, Gherardi E. Signalling by HGF/SF and Met: the role of heparan sulphate co-receptors. Biochem Soc Trans. 2006;34(Pt 3):414–7. doi:10.1042/BST0340414.
130. Ozen E, Gozukizil A, Erdal E, Uren A, Bottaro DP, Atabey N. Heparin inhibits hepatocyte growth factor induced motility and invasion of hepatocellular carcinoma cells through early growth response protein 1. PLoS One. 2012;7(8):e42717. doi:10.1371/journal.pone.0042717.
131. Iscan E, Gunes A, Korhan P, Yilmaz Y, Erdal E, Atabey N. The regulatory role of heparin on c-Met signaling in hepatocellular carcinoma cells. J Cell Commun Signal. 2016; doi:10.1007/s12079-016-0368-0.