Liver Stem Cells and Molecular Signaling Pathways in Hepatocellular Carcinoma

Krit Kitisin, Michael J. Pishvaian, Lynt B. Johnson, Lopa Mishra

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most lethal cancers. Surgical intervention is the only curative option, with only a small fraction of patients being eligible. Conventional chemotherapy and radiotherapy have not been effective in treating this disease, thus leaving patients with an extremely poor prognosis. In viral, alcoholic, and other chronic hepatitis, it has been shown that there is an activation of the progenitor/stem cell population, which has been found to reside in the canals of Hering. In fact, the degree of inflammation and the disease stage have been correlated with the degree of activation. Dysregulation of key regulatory signaling pathways such as transforming growth factor-beta/transforming growth factor-beta receptor (TGF-β/TBR), insulin-like growth factor/IGF-1 receptor (IGF/IGF-1R), hepatocyte growth factor (HGF/MET), Wnt/β-catenin/FZD, and transforming growth factor-α/epidermal growth factor receptor (TGF-α/EGFR) in this progenitor/stem cell population could give rise to HCC. Further understanding of these key signaling pathways and the molecular and genetic alterations associated with HCC could provide major advances in new therapeutic and diagnostic modalities.

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Hepatocellular carcinoma (HCC) is the fifth most common solid malignancy worldwide and causes more than 600,000 deaths annually.[1] Current data indicate that the incidence of HCC is steadily increasing in the United States.[2,3] Prognosis remains extremely poor with a 5-year survival rate of less than 5% without treatment.[3] Currently, the only curative therapeutic option for early-stage HCC is surgical intervention, including percutaneous ablation, hepatic resection, and liver transplantation. However, only 12% of diagnosed HCC patients are deemed eligible for curative therapy.[4,5]

Accumulating evidence suggests that development of HCC is a multistep process associated with changes in host gene expression, altered DNA methylation, and point mutations or loss of heterozygosity (LOH) in selected cellular genes.[6] The dynamics of these cellular changes remain unclear, and it is still a challenge to identify the rate-limiting steps in initiation and progression of HCC. However, a number of molecular changes occur in high frequency in pre-neoplastic tissues, such as cirrhotic tissue, hepatic adenomas and dysplastic nodules. For example, chronic hepatitis B virus (HBV) infection, which is one of the most prominent risk factors for hepatocarcinogenesis, appears to disrupt senescence-related pathways by different mechanisms. These include inactivation of p53, $p55^{sen}$ and hyperphosphorylation of the retinoblastoma protein (pRb), as well as down-regulation of sui1 (a translational factor) and the cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1/SDI1}$.[7–9] Perturbation of several signaling pathways such as wingless (Wnt/β-catenin/FZD), JAK/STAT, MAPK, insulin-like growth factor 2 (IGF-2), and transforming growth factor-beta (TGF-β) have also been identified.[10] Approximately 40% of HCCs display chromosomal abnormalities.[11–14] In addition, microsatellite instability (MSI) and dysfunction of the mismatch repair genes, hMSH2 and hMLH1, are present in up to 11% of HCCs.[15,16] These are in turn associated with mutations in TGFβRII, M6P/IGFIIR, and BAX genes.[17] Among proto-oncogenes, c-myc is upregulated in approximately 50% of HCCs,[18,19] and cyclin D1 is overexpressed in approximately 40% of HCCs.[20,21] Dysregulation of these positive mediators of cellular proliferation promotes autonomous and unregulated cellular growth in HCC.

STEM CELLS AND HEPATOCARCINOGENESIS

Both epigenetic and genomic alterations that accumulate in prolonged chronic inflammatory states, such as in cirrhosis and chronic hepatitis, compromise an intricate balance of various regulatory pathways, resulting in accelerated proliferation of

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hepatocytes and development of monoclonal hepatocyte populations. These populations harbor dysplastic hepatocytes that will eventually evolve to dysplastic nodules, which are true pre-neoplastic lesions, 30% of which will develop into HCCs within 5 years.[22] Though these dysplastic lesions derive from monoclonal cell populations, different lesions from the same liver possess different combinations of genomic aberrations. This heterogeneity in genomic changes supports the view that diverse combinations of cellular alterations can sufficiently transform normal hepatocytes into malignant ones.

In continuously renewing systems such as gastrointestinal, hematopoietic, and epidermal tissues, it is widely accepted that stem cells are the only cells with the potential to acquire sufficient genetic alterations for malignant transformation because of their long lifespan as compared to short-lived differentiated cells. However, the liver has several cell types that have the longevity to acquire the requisite number of genetic changes for neoplastic development. Hepatocytes, cholangiocytes, and progenitor/stem cells are normally relatively dormant, but they possess enormous proliferative capacity under certain stimuli. In rodents, the liver can be restored to its original volume within 10 days even after a two-thirds partial hepatectomy.[23,24] Transplantation of normal mature wild-type hepatocytes into transgenic mice with urokinase plasminogen activator, in which the transgenic hepatocytes undergo massive necrosis, results in 12 or more cell divisions and most of the transgenic liver is replaced.[25] Serial transplantation experiments also have shown a near infinite proliferative capacity of hepatocytes.[26] It has also been shown that when there is massive bile duct injury, hepatocytes can differentiate into cholangiocytes.[27] Thus, mature hepatocytes have stem cell properties. However, in prolonged chronic inflammatory diseases, such as viral hepatitis and cirrhosis, hepatocytes actually undergo replicative senescence because of telomerase shortening.[26] Another cell population, a progenitor/stem cell, is activated under these circumstances and it is thus possible that progenitor/stem cells could be at least partly responsible for HCCs.

In general, stem cells are undifferentiated cells with the ability to undergo asymmetric cell division producing two different daughter cells: a stem cell and a committed progenitor cell. In the adult gastrointestinal system, stem cells generally considered to be tissue-specific are able to give rise only to progeny cells corresponding to their tissue of origin. During liver development, the liver bud is seen around embryonic day (ED) 8.5 as endodermal stem cells begin to proliferate and differentiate under the influence of fibroblast growth factor signaling from the cardiogenic mesoderm[28] and bone morphogenetic protein signaling from the septum transversum mesenchyme.[29] This region then produces cells destined to become hepatoblasts, bipotential cells committed to fetal hepatocytes, and biliary epithelial cells. Hepatoblasts begin expressing alpha-fetoprotein (AFP) and albumin (Alb) and later express cytokeratins (CKs) 7 and 19. Just before ED16, hepatoblasts diverge along two cell lineages: hepatocytes (AFP^+/Alb^+) and cholangiocytes ($CK19^+$).[30,31] In adult human tissues, these hepatic progenitor cells (or mouse oval cells) are immature epithelial cells found residing in the smallest terminal branches of the biliary tree called the canals of Hering.[32] This compartment is also known as the progenitor cell compartment in humans and oval cell compartment in rodents.[33] In this compartment, the immature epithelial cells, which express both bile ductular and hepatocyte markers (CK19 and AFP), are in direct physical continuity with hepatocytes at one membrane boundary and bile duct cells at another boundary and are considered to represent hepatic stem cells.[34,35]

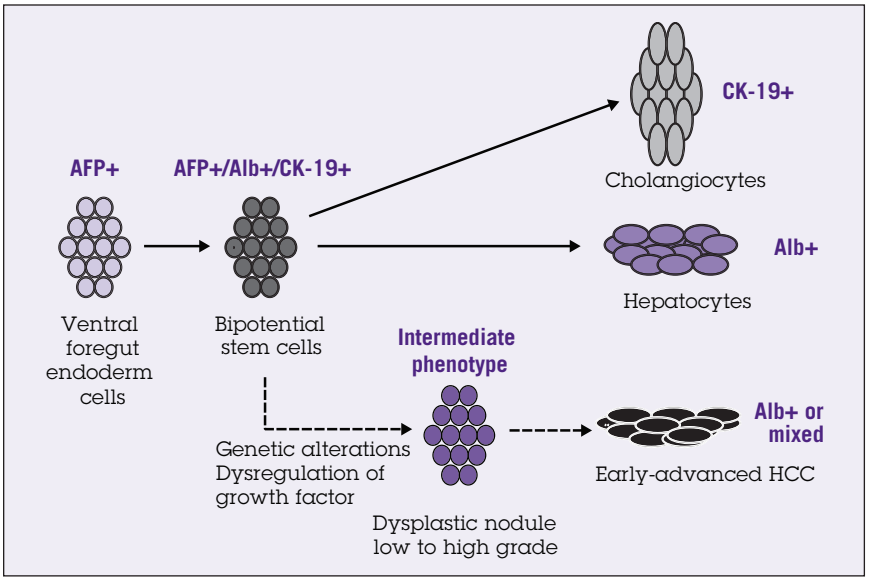

Figure 1. Schematic representation of fetal liver development and hepatocarcinogenesis process based on stem cell model. About half of the small cell dysplasic lesions consist of progenitor cells and intermediate cells. Abbreviations: AFP = alpha-fetoprotein; Alb = albumin; HCC = hepatocellular carcinoma.

The progenitor cell compartment (oval cell compartment in rodents) can be activated when the mature hepatocytic or cholangiocytic compartments are damaged or their replication is inhibited.[32,36] Such circumstances can be observed in cirrhosis and chronic inflammatory liver diseases when hepatocytes undergo senescence owing to telomere shortening after 20 to 30 years of continuous replication.[26] Activation of the progenitor cell compartment, also known as "ductular reaction" ("oval cell reaction" in rodents), is merely an expansion of transit amplifying progenitor cells, which can differentiate into hepatocytes and biliary cells. Intermediate hepatocytes, which have an intermediate phenotype between progenitor cells/biliary ductular cells and mature hepatocytes, are seen in moderate-to-severe inflammatory hepatitis. In fact, the degree of progenitor cell activation and the number of these intermediate hepatocytes correlate with the degree of inflammation and fibrosis in diseases like chronic hepatitis, hemochromatosis, and non-alcoholic steatohepatitis.[37,38] It has been demonstrated that sequestered hepatocytes in cirrhosis are in continuity with reactive ductules.[39]

Several studies have shown a progenitor cell phenotype in a substantial number

of HCCs. Detailed immunophenotyping of HCCs revealed that 28% to 50% of HCCs express markers of progenitor/biliary cells such as CK7 and CK19.[40–43] These tumors also consist of cells that have an intermediate phenotype between progenitors and mature hepatocytes. In fact, HCCs with CK19 expression have a significantly worse prognosis and higher recurrence after surgical resection and liver transplantation than CK19-negative HCCs.[40] Nevertheless, the question remains whether this immature intermediate phenotype represents progenitor cell differentiation arrest or dedifferentiation of mature hepatocytes. The histologic and immunophenotyping studies favor the progenitor cell differentiation arrest model. Small dysplastic foci (less than 1 mm in size) represent the earliest premalignant lesions, and 55% of them are comprised of progenitor cells and intermediate hepatocytes, instead of senescent hepatocytes.[26] A recent study also identified a side population of cells (SP), which have characteristics of both hepatocytic and cholangiocytic lineages, in human HCC cell lines, Huh7 and PLC/PRF/5. Importantly, injection of SP cells into nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice leads to tumor formation. Tumor-initiating potential is maintained in these SP cells after serial transplantations.[44] These results suggest that hepatic progenitor/stem cells could account for human hepatocarcinogenesis (Figure 1).

MOLECULAR SIGNALING PATHWAYS AND HEPATOCARCINOGENESIS

Normal embryogenesis and organ development as well as tissue regeneration and repair require an intricate balance of various molecular growth factor signals. Dysregulation of these signaling pathways and their components is the central principle in human tumorigenesis. Recent studies have identified regulatory pathways including TGF-β/TBR, IGF/IGF-1R, HGF/MET, Wnt/β-catenin/FZD, and TGF-α/EGFR as key contributory factors to the transformation, proliferation, antiapoptosis, and invasive behaviors of human HCCs.

Transforming Growth Factor-Beta Signaling

Initially described for its transforming capability, TGF-β plays an important role in a wide range of cellular responses, including cellular homeostatsis, cell differentiation, proliferation, migration, and apoptosis. The TGF-β superfamily comprises more than 30 members, including the TGF-βs, bone morphogenetic proteins, activins, and nodal. The basic signaling cascade of TGF-β involves type I and type II transmembrane serine/threonine kinase receptors (TBRI and TBRII). Cellular responses are mediated by the intracellular signaling proteins, Smads.[45] Vertebrates possess at least nine Smad proteins categorized into three functional classes: (1) receptor activated Smads (R-Smads): Smad1, Smad2, Smad3, Smad5, and Smad8; (2) co-mediator Smads: Smad4 and Smad10; and (3) inhibitory Smads: Smad6 and Smad7.[46] Activation of Smads by TGF-β results in association of R-Smads with Smad4. This Smad complex then translocates to the nucleus where it participates in regulation of TGF-β target gene expression such as p15, p21, and E-cadherin by interacting with various transcriptional factors such as CBP/p300 and SKI/SNO[47,48] (Figure 2).

Recent studies show that adaptor proteins such as embryonic liver fodrin protein (ELF), the Smad anchor for receptor activation (SARA), filamin, and microtubules play critical roles in modulating TGF-β signaling. Genetic studies have highlighted that ELF, a major dynamic scaffolding protein, is required for Smad3 and Smad4 co-localization.[49-51] Disruption of ELF results in mislocalization of Smad3 and Smad4 leading to loss of the TGF-β–dependent transcriptional response.[49,52,53] Interestingly, we have also demonstrated in previous work that ELF is crucial for multiple developmental processes. ELF$^{-/-}$ mutant mice share strikingly similar phenotypes with double heterozygous Smad2/Smad3 mutants, including profound defects in gut, liver, and cardiovascular and nervous systems.[54-58] Livers from these mutants display distorted liver architecture and scant early intrahepatic bile duct development.[49] Interestingly, by 15 months of age, 40% of heterozygous ELF mice spontaneously developed HCC and also showed additional phenotypic changes such as increased centrilobular steatosis and high-grade dysplasia. A marked attenuation of the TGF-β–mediated antiproliferative response has also been shown in several human HCC cell lines.[53] Functional inactivation of TGF-β signaling via expression of a dominant-negative mutant TBRII in transgenic mice treated with the carcinogen diethylnitrosamine resulted in higher rates of pre-neoplastic lesions and HCCs as compared with similarly treated wild-type mice.[6] In addition, MSI or inactivation

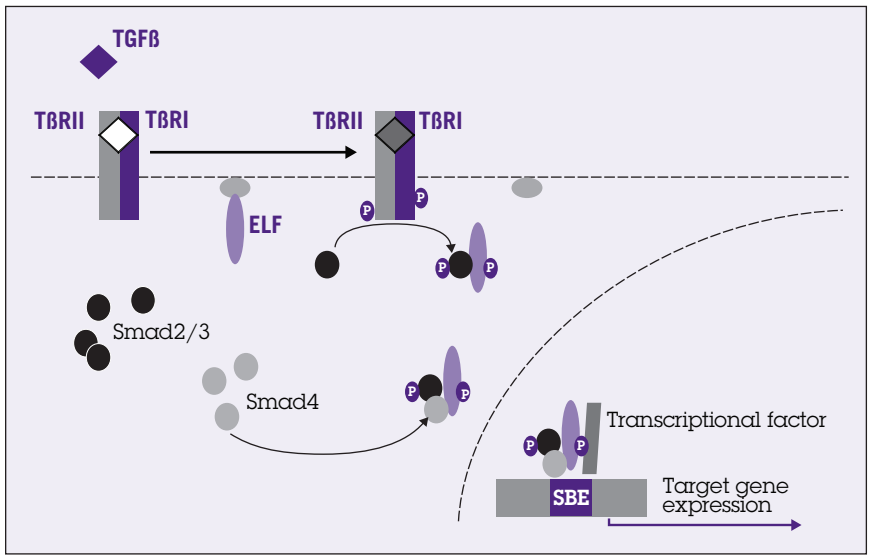

Figure 2. Transforming growth factor-beta (TGF-β) signals through distinct receptors and Smads, which are modulated by β-spectrin embryonic liver fodrin protein (ELF). TGF-β binds to serine/threonine kinase receptor complexes I and II (TBRI and TBRII), which subsequently phosphorylates receptor-associated Smad proteins, such as Smad2 and Smad3. Smad2/3 then forms heterometric complex with Smad4 and ELF proteins and translocates to the nucleus, interacting with transcriptional factor and activating target genes. Abbreviation: SBE = Smad binding element.

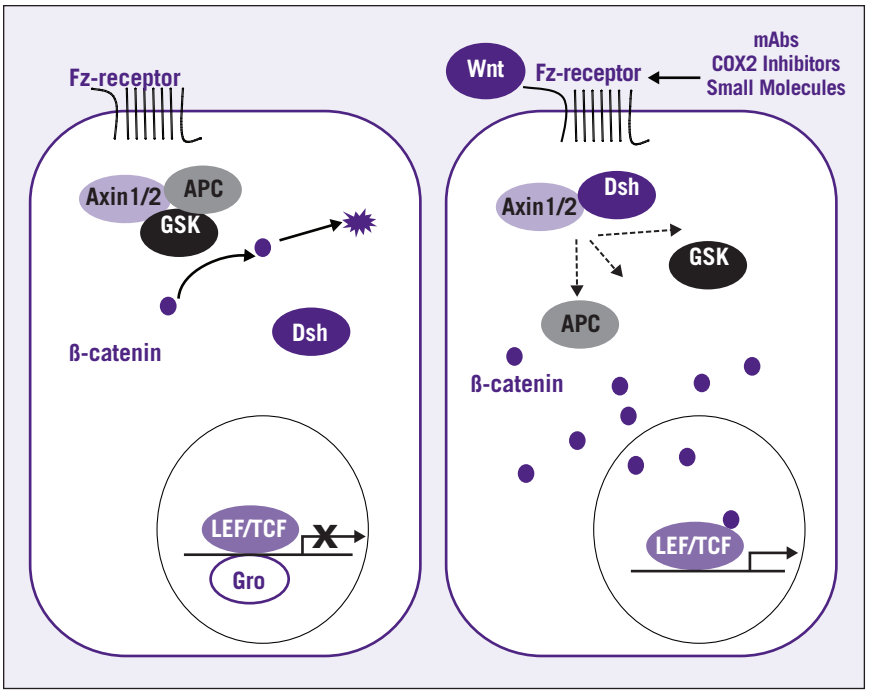

Figure 3. In the absence of Wnt stimulation, the APC (adenomatous polyposis coli) forms a trimeric complex, known as the "destruction complex," with glycogen synthase kinase-3β (GSK) and Axin. This complex then interacts with β-catenin and degrades by the ubiquitin-proteasome pathway. When Wnt ligands bind to the seven-transmembrane receptor, the cytoplasmic protein Dishevelled (Dsh) is recruited to the membrane and binds to Axin1 and Axin2. The mechanism of Dsh-mediated inhibition of Axin is not well understood, but it has been suggested that Dsh might disrupt the destruction complex. Inhibition of Axin results in accumulation of β-catenin, which subsequently translocates into the nucleus. β-catenin interacts with LEF/TCF (lymphocyte enhancer factor/T cell factor) proteins and serves as a coactivator of LEF/TCFs to stimulate transcription of Wnt target gene. Grouch protein (Gro) acts as corepressor of LEF/TCFs and normally binds to LEF/TCFs in the absence of β-catenin. New therapeutic treatments aimed at this pathway include monoclonal antibodies, cyclooxygenase (COX)-2 inhibitors, and several small molecules.

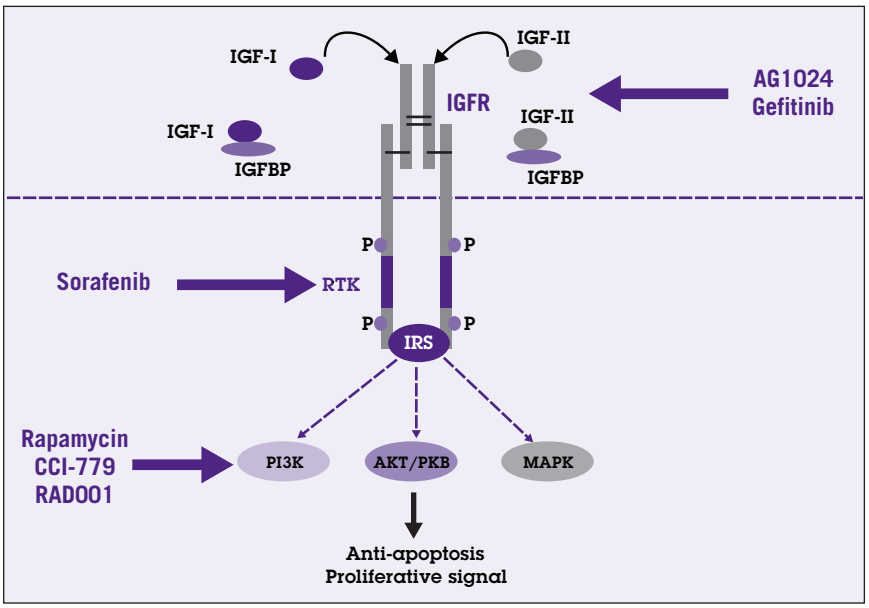

Figure 4. Simplified schematic diagram of insulin-like growth factor (IGF) signaling. IGF-I and IGF-II bind to IGF receptor (IGFR), a receptor tyrosine kinase (RTK), with high affinity resulting in phosphorylation of intracellular proteins including insulin receptor substrate (IRS). The signal is then conveyed to specific downstream effectors such as phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT/PKB), and mitogen-activated protein kinase (MAPK) pathways. These pathways play crucial roles in antiapoptosis as well as cell proliferation. Bioavailability of both IGFs is influenced by the presence of IGF binding proteins (IGFBP). New therapeutic agents such as AG1024 and gefitinib aim to block this signaling pathway at level of the receptor. Downstream targeting agents such as rapamycin, CCI-779, and RAD001 are also under investigation.

of the mismatch repair genes, hMSH2 and hMLH1, is present in up to 11% of HCCs.[15,16] and is in turn associated with mutations in TGFβRII, M6P/IGFIIR, and BAX genes.[17] Most studies document a reduction of TGF-β receptors in up to 70% of HCCs.[59] However, Smad proteins shown to be impaired in other cancers appear to play a minor role in HCCs. Smad4, which is mutated in 50% of pancreatic cancers, is also mutated in 10% of HCCs. Similarly, Smad2 mutations are identified in fewer than 5% of HCCs.[60,61] Finally, inhibitory Smad7 is upregulated in 60% of advanced HCCs.[62]

Yet, TGF-β levels in serum and urine are increased in HCC patients.[63,64] In addition, up to 40% of HCCs have increased TGF-β based on immunohistochemical studies.[65,66] High TGF-β expression levels have been correlated with advanced clinical stages of HCC.[67] The dual role of TGF-β signaling in HCCs may be explained by its effect on the tumor tissue microenvironment and on selective loss of the TGF-β–induced antiproliferative pathway. Tumor-derived TGF-β could contribute to tumor growth indirectly by suppressing immune surveillance or stimulating production of angiogenic factors. Tumor cells that have selectively lost their growth-inhibitory responsiveness to TGF-β but retain an otherwise functional TGF-β signaling pathway may exhibit enhanced migration and invasive behavior in response to TGF-β stimulation.[68,69] TGF-β signaling also has been shown to induce an epithelial to mesenchymal transition (EMT) in these cells.[69] This EMT process is characterized by decreased cell-cell adhesion, through the decrease in E-cadherin, leading to enhanced migration and invasiveness.

Wingless/β-Catenin Signaling

The Wnt signaling pathway is highly conserved evolutionarily. It plays an important role in cell proliferation, cell/cell interactions, motility, tissue development and modeling, as well as axis formation.[70,71] Initiation of Wnt signaling involves the binding of Wnt proteins to a receptor complex consisting of the frizzled receptor family (Fz) and a member of the low-density lipid receptor family, Lrp5 or Lrp6. The key intracellular component is cytoplasmic β-catenin protein.[72,73] Under normal circum-

stances, when the Wnt signaling pathway is inactive, the tumor suppressor APC forms a trimeric complex, known as the "destruction complex," with glycogen synthase kinase-3β (GSK3β) and axin/conductin. This complex interacts with and serine phosphorylates β-catenin, thus targeting it for degradation by the ubiquitin-proteasome pathway. In addition to this canonical degradation, β-catenin can be degraded without phosphorylation through a pathway involving a p53-inducible E3-ubiquitin ligase, seven in absentia homolog (SIAH).[74] When Wnt ligand is present, it binds to Fz and Lrp5 or Lrp6. Ligand binding results in accumulation of cytoplasmic β-catenin by recruiting the cytoplasmic protein Dishevelled (Dsh), which disrupts the destruction complex. β-catenin then interacts with a member of the TCF/LEF (T-cell factor/lymphocyte enhancer factor) family of DNA-binding proteins, resulting in activation of Wnt target genes to increase cell proliferation.[75,76] Wnt/β-catenin target genes include cell-cycle promoting genes such as c-MYC and cyclin D1, the anti-apoptotic gene survivin, and pro-invasive genes such as MMP.[59] Therefore, a mutation in Wnt signaling pathway components results in the accumulation of β-catenin, and predisposes tissues to tumorigenesis (Figure 3).

Alterations in Wnt signaling components have been described in HCCs. Up to 40% of HCCs exhibit accumulation of nuclear β-catenin. Several mechanisms contribute to β-catenin accumulation in HCC, such as downregulation of the Dsh-inhibitor, dapper homolog 1 (HDPR1), and upregulation of PIN1 (a prolyl cis/trans isomerase), a β-catenin/APC destabilizer.[77-79] Axin-2 has been found to be mutationally inactivated in 3% to 14% of HCCs.[80,81] In addition, the Fz-7 receptor is frequently overexpressed.[82] Taken together, these results suggest that more than one portion of the Wnt signaling pathway has to be dysregulated in liver tumors to achieve aberrant β-catenin nuclear accumulation.

Insulin-Like Growth Factor Signaling

IGF signaling plays a central role in embryogenesis, lifespan regulation, and cell proliferation. IGF signaling consists of IGF ligands (IGF-I and IGF-II), IGF binding proteins (IGFBP 1-6), and membrane-bound IGF receptors (IGF-1R, IGF-II/M6PR, and IGF-2R). IGF ligands can also bind to the insulin receptor. IGF signaling is initiated by the binding of IGF ligands, which results in phosphorylation of intracellular target proteins. These proteins then convey the signal to specific downstream effectors such as INSR-substrate IR5 leading to activation of, for example, phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT/PKB). Binding of growth factor receptor-bound protein 2 (Grb2) to the receptor can lead to activation of mitogen-activated protein kinase family (MAPK) signaling. The result is transcriptional activation of various target genes such as p27, c-myc, c-FOS, cyclin B and vascular endothelial growth factor (VEGF)[83] (Figure 4).

Dysregulation of IGF signaling in HCC occurs predominantly at the level of IGF-II bioavailbility. Overall, increased levels of IGF-II are found in 16% to 40% of HCCs.[59] In chronic viral hepatitis B, HBV-derived HBx protein contributes to overexpression of IGF-II through SP1-mediated reactivation of fetal-type IGF-II.[84,85] In chronic hepatitis C, the HCV-derived core gene product also induces overexpression of IGF-II by acting as a transactivator through SP1 and EGR-1 binding sites.[86] IGF-II bioavailability is inversely dependent on IGF-2R expression level. A reduced IGF-2R level is associated with less ligand-receptor binding and thus a relative increase in local IGF-II bioavailability. In fact, IGF-2R level is reduced in 63% of HCCs[87] and loss of heterozygosity at the IGF-2R locus has been implicated in HCCs and in pre-neoplastic lesions.[88,89] Similarly, reduced expression of IGFBP also results in a relative increase in IGF-II concentration.

Hepatocyte Growth Factor/MET Signaling

HGF is one of the most potent growth factors for hepatocytes and plays crucial roles in proliferation, migration, cell survival, morphogenesis, angiogenesis, and tissue regeneration.[90] HGF binds the tyrosine kinase receptor, c-MET, which is expressed in epithelial and endothelial cells. Binding of HGF to the c-MET receptor results in receptor autophosphorylation as well as phosphorylation of adaptor proteins such as Gab-1 and Grb2. The HGF signaling is then conveyed to activation of various downstream effectors such as phospholipase C (PLC), Stats, PI3K, and extracellular signaling-regulated kinase (ERK1/2).[91] Specificity of HGF signaling is achieved through different membranous binding partners and the adaptor proteins. Examples of HGF target genes are MMP and urokinase-type plasminogen activator (uPA) (Figure 5).

HCC has been shown to release tumor cell products inducing stellate cells and myofibroblasts to secrete HGF. The increased HGF from these cells in turn promotes tumor cell invasiveness.[92-94] Furthermore, c-MET is also

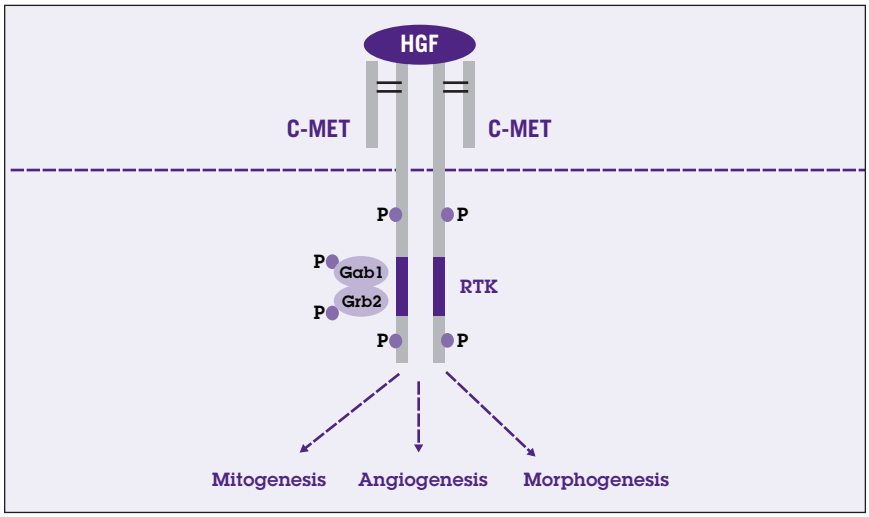

Figure 5. Simplified schematic of hepatocyte growth factor (HGF)/MET signaling pathway. Activation of phosphorylation of the kinase domain in cMET, a receptor tyrosine kinase (RTK), by HGF results in activation of adaptor proteins: growth factor receptor-bound protein2 (Grb2) and Grb2-associated binding protein (Grb2 and Gab1). Activation of these adaptor proteins leads to activation of various downstream effectors resulting in transcription of various genes important in mitogenesis, angiogenesis, and morphogenesis.

overexpressed in HCC as compared with normal liver. c-MET is overexpressed in 20% to 48% of HCCs. The increase in c-MET could be partly due to genomic alterations such as 7q gains or to growth factor-dependent transcriptional activation of c-MET.[95–97] However, increased c-MET levels do not appear to correlate with tumor size or invasiveness.[96]

Transforming Growth Factor-α/Epidermal Growth Factor Signaling

TGF-α/EGF signaling comprises at least eight ligands including TGF-α, EGF, heparin-binding EGF, amphiregulin, betacellulin, epiregulin, epigen, and crypto. TGF-α/EGF signaling conveys its signal through the receptor tyrosine kinase family: EGFR (Her1/ErbB-1), neu/Her2/ErbB-2, Her3/ErbB-3, and Her4/ErbB-4. Different ligand specificities and concentrations lead to differential phosphorylation of multiple tyrosine residues at the cytoplasmic portion of the molecule.[98] Phosphorylation of the receptor's cytoplasmic portion serves as a docking site for recruitment of proteins with Src homology 2 domains, such as Grb2 and Shc.[99] The signal will then activate multiple downstream pathways, which can directly or indirectly interact with each other (Figure 6).

TGF-α and EGF act as potent mitogens for hepatocytes and stimulate DNA synthesis.[100] TGF-α has been shown to be overexpressed in human HCCs as well as in HCC cell lines.[101] TGF-α appears to act during the early stages of hepatocarcinogenesis and its level is correlated with tumor differentiation and proliferation.[102,103] The pro-TGF-α/EGF signaling level is increased from normal liver to preneoplastic lesions and to HCCs. Interestingly, TGF-α levels in HCCs correlate with the presence of viral polypeptides (HBS and HBC) in the adjacent non-cancerous liver tissue; and in HCC cells, HBV-DNA induces TGF-α expression.[104] Heparin-binding EGF, which can be used as a prognostic marker for disease-free survival, is also markedly increased in 59% to 100% of HCCs as compared with surrounding normal tissue by immunohistochemical staining.[105]

NEW THERAPEUTIC TARGETS

The only curative options for earlier stages of HCC are surgical resection, transplantation, or percutaneous ablation. However, only 12% of patients are eligible for surgical intervention because most HCCs are diagnosed at a late stage and the majority develop in diseased livers with poor hepatic functional reserve. Numerous experimental strategies are aimed at the aforementioned growth factor molecular pathways. A major signaling pathway in HCC is TGF-β, and serine/threonine kinase inhibitors (RSTK) have been developed. SB-431542, a small RSTK, has been shown to block TGF-β– and activin-mediated signaling. Reduced phosphorylation and decreased nuclear translocation of the Smads are observed with this inhibitor. More important, TGF-β–evoked protumorigenic cellular effects are diminished.[106] SD-208 RSTK has also been shown to inhibit TGF-β–mediated migration and invasion in tumor cell models. However, viability and proliferation are not decreased with this compound.[107]

Therapeutic options targeting Wnt signaling are limited; however, receptor tyrosine kinase inhibitors (RTK) have also been shown to reduce receptor signaling. The crucial consideration is to create a highly selective inhibitor that will not interfere with other receptor tyrosine kinases such as INSR, which can lead to diabetogenic effects. Highly selective inhibitors such as tyrphostins, NVP-AEW541, and cycloligninans are able to reduce activation of IGF-1R and downstream AKT/PKB. In vitro and in vivo studies have shown a reduction in tumor cell growth especially in combination with chemotherapeutic agents.[108,109] Novel neutralizing antibodies against IGF-1R such as IR3 which has been shown to reduce receptor autophosphorylation and signaling are also under active investigation.[110–112] Antisense RNA and antisense oligodeoxynucleotide techniques to block IGF-1R synthesis are another approach to increase apoptosis and reduce proliferation.[113]

Inhibitors of the c-MET receptor such as PHA-665752, SU11271, SU11274, and SU11606 have been shown to reduce c-MET phosphorylation, resulting in subsequent inactivation of downstream effectors such as AKT/PKB, Gab-1, PLC, and Stat3. By blocking the c-MET receptor, decreases in proliferation, cell motility, and invasion of different types of tumor cells have been achieved. In a tumor xenograft mouse model, PHA-665752 caused a reduction in tumor volume and intensive cell death.[114] Treatment of HCC with SU5416, an RTK inhibitor, was also shown to reduce activation of ERK1/2 and AKT/PKB effectors. The Ras/VEGF-R inhibitor BAY 43-9006 (sorafenib) reduced proliferation and angiogenesis in a promising phase II clini-

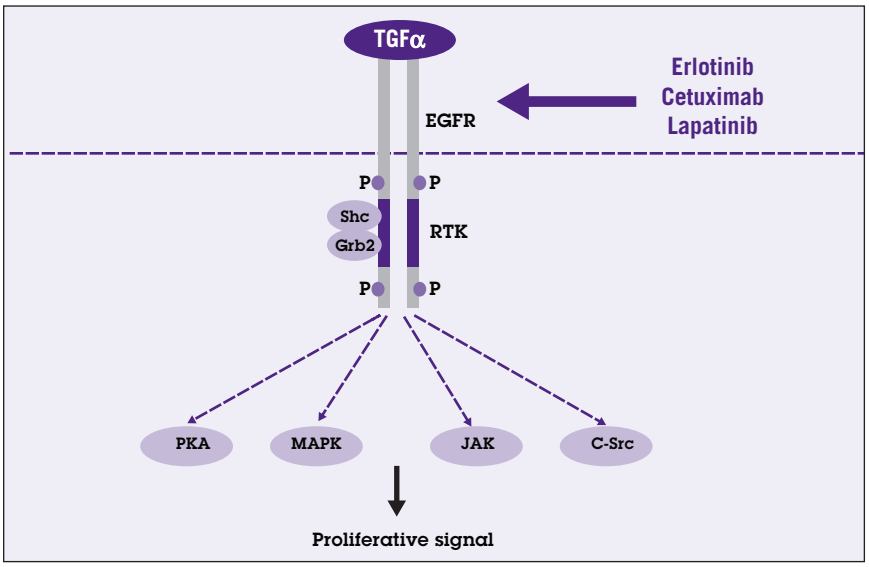

Figure 6. Simplified schematic diagram of transforming growth factor-alpha/epidermal growth factor receptor signaling pathway (TGF-α/EGFR). TGF-α binding to EGFR results in stimulation of the endogenous receptor tyrosine kinase (RTK). The activated membrane-bound EGFR serves as a docking site for recruitment of proteins such as Src homology 2 domain containing (Shc) and growth factor receptor-bound protein2 (Grb2). The signal then activates one of several intracellular signal transduction pathways including protein kinase A (PKA), mitogen-activated protein kinase (MAPK), Jak/Stat, and C-src pathways, which play important roles in cell proliferation. New therapeutic agents such as erlotinib, cetuximab, and lapatinib aim to block this signaling cascade.

cal trial. Treatment of HCC patients with BAY 43-9006 resulted in stable disease or tumor shrinkage in 43% of cases.[59]

CONCLUSIONS

Emerging studies regarding the role of hepatic progenitor/stem cells are only now being described. By far, the major underlying etiology of HCCs is chronic inflammatory diseases from viral hepatitis or cirrhosis. Under these circumstances, the progenitor cell compartment is activated as the mature hepatocytes become senescent. Substantial numbers of HCCs display an intermediate phenotype of progenitor and hepatocyte cells. Current studies favor the differentiation arrest model, in which the maturation process of the progenitor/stem cell is disrupted. Many growth factor signaling pathways play crucial roles in the maturation of liver cells such as TGF-β/TBR, IGF/IGF-1R, HGF/MET, Wnt/β-catenin/FZD, and TGF-α/EGFR signaling. Dysregulation of these pathways represents a crucial role in hepatocarcinogenesis. Further studies are necessary to elucidate cross-talk between the different regulatory pathways. The understanding of these interdependent regulatory pathways holds promise for the development of new therapeutic approaches to this devastating disease.

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Dr. Mishra has no potential conflicts of interest to disclose.