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Signaling Pathways in Liver Cancer

Xinle Wu and Yang Li
Amgen Inc.
USA

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world, with approximately 630,000 new cases each year (Alves et al., 2011). It is also the third most common cause of cancer related mortality (Parkin et al., 2005). Moreover, the incidence of HCC has risen in many countries over the past decade. The greatest risk factor associated with the development of HCC is hepatitis B and C virus infection. Hepatitis infection is believed to increase the risk of developing HCC by 20 fold and is the major etiological factor in more than 80% of HCC cases (Anzola, 2004). Other main risk factors include excessive alcohol consumption, non alcoholic steatohepatitis, exposure to environmental toxins such as aflatoxin B, hemochromatosis, cirrhosis, diabetes and obesity (El-Serag et al., 2006; Hassan et al., 2010; Whittaker et al., 2010).

The standard treatments for early stage HCC, such as surgical resection and liver transplantation, can cure certain population of patients. However, due to the asymptomatic nature of early HCC and lack of effective screening strategies, 80% of patients present with advanced HCC at the time of diagnosis (Thomas and Abbruzzese, 2005). These patients have rather limited treatment options which are mainly palliative. If the patient has developed HCC that is surgical unresectable, systemic treatments are commonly used. However, conventional chemotherapy with cytotoxic agents is not very effective in changing the progress of the tumor growth. Consequently, the mortality rate for advanced stage HCC is quite high, and 5 year survival rate for patients with HCC is only 7% (Bosch et al., 2004; Whittaker et al., 2010). There is clearly an urgent medical need to develop new effective and well-tolerated HCC treatment options.

In recent decades, tremendous progress has been made toward better understanding the molecular mechanisms of oncogenic processes. Many cell signaling pathways involved in tumor pathogenesis have been identified, leading to the identification of new molecular targets for therapeutic development. Unlike conventional chemotherapy which utilizes broad spectrum cytotoxic agents, targeted therapy acts directly and specifically on the components that regulate tumorigenesis. This strategy has shown clinical benefits in various tumor types, such as breast, colorectal and lung cancers (Ansari et al., 2009; Slamon et al., 2001; Takahashi and Nishioka, 2011). Several major cellular signaling pathways have been implicated in HCC, and below we will review those pathways and discuss the potential to target the molecular components within those signaling pathways.

2. Major signaling pathways

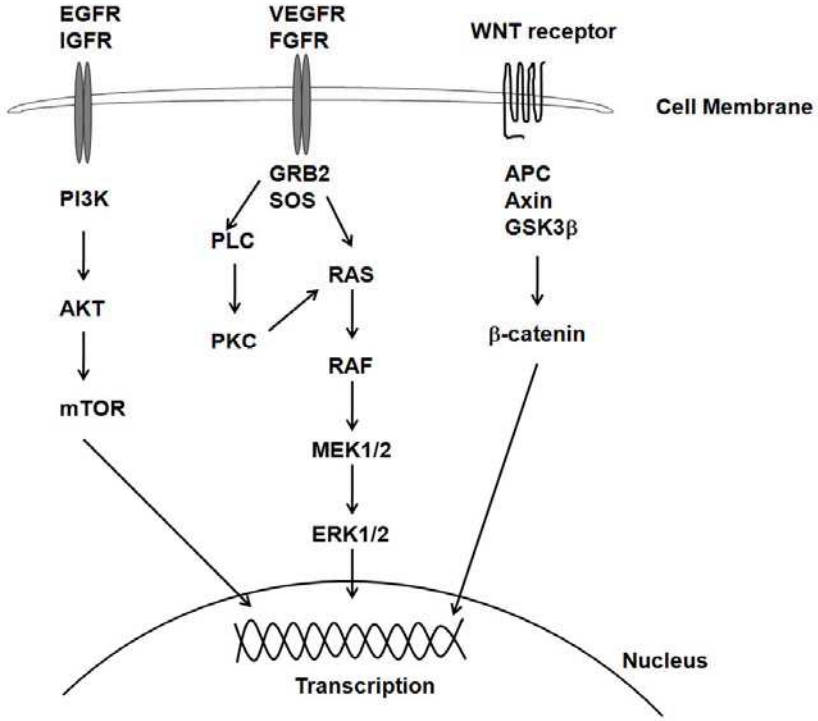

Fig. 1. Major cellular signaling pathways involved in hepatocellular carcinoma tumorigenesis

2.1 Wnt/β-catenin pathway

Wnt signaling pathway involves three main components which include the cell surface, the cytoplasm, and the nucleus (Giles et al., 2003). At the cell membrane, Wnt ligands form complexes with Frizzled receptors and LRP5 or LRP6 co-receptors (Dale, 1998; Wehrli et al., 2000). There are at least 19 known human Wnt family genes and 10 Frizzled family genes, and most Wnt proteins can bind to multiple Frizzled receptors and vice versa (Wang et al., 1996). This ligand receptor interaction can be additionally regulated by other secreted factors and cell surface proteins. For example, soluble forms of Frizzled proteins compete with cell membrane Frizzled receptor for Wnt ligands (Finch et al., 1997). And DKK proteins block Wnt signaling by binding to LRP6 co-receptor (Fedi et al., 1999).

In the cytoplasm, β-catenin associates with a multiprotein complex which includes tumor suppressor proteins APC, axin, and Ser/Thr kinase GSK3β. Axin and APC form a structural scaffold that allows GSK3 β to phosphorylate β-catenin. Phosphorylation of β-catenin targets it for ubiquitination and protein degradation (Clevers, 2006; Gordon and Nusse, 2006). Therefore, under basal conditions, in the absence of Wnt ligands, β-catenin is continuously

degraded in the cytosol. Upon Wnt binding, axin is recruited to the membrane to LPR5 and the β-catenin destruction complex is then inactivated. This allows the unphosphorylated β-catenin to accumulate and to translocate into the nucleus (Clevers, 2006; Gordon and Nusse, 2006). The nuclear β-catenin then forms a complex with TCF/LEF family of DNA binding transcription factors to activate TCF/LEF target genes. Many of the target genes are involved in cell proliferation, such as cyclin D1 (Shtutman et al., 1999).

Besides regulating gene transcription, β-catenin also participates in cell–cell adhesion. Cell-cell adhesion and separation are important physiological processes involved in development as well as tumor metastasis. It is known that cadherin mediated adhesion is regulated by β-catenin. The formation of stable cell–cell adhesions depends on the integrity of a core complex including E-cadherin, β-catenin and α-catenin. β-catenin binds directly to the cytoplasmic domain of E-cadherin and such association is regulated by the phosphorylation state of β-catenin (Nelson and Nusse, 2004).

Wnt signaling clearly plays important roles in normal liver function as 8 out of the total 19 Wnt ligands are expressed in hepatocytes (Thompson and Monga, 2007). Both LRP5 and LRP6 and 9 of 10 Frizzled receptors are expressed in normal liver (Fujino et al., 2003; Zeng et al., 2007). Evidence has suggested that the Wnt signaling pathway is critically involved in liver development and postnatal liver homeostasis. In addition, this pathway is also associated with many other important liver functions, such as ammonia and nitrogen metabolism, bile acid homeostasis, drug detoxification and injury recovery (Behari, 2010; Takigawa and Brown, 2008).

Multiple lines of evidence link aberrant Wnt signaling to HCC. Wnt signaling pathway activation is frequently reported in liver carcinoma. It has been demonstrated that there are significant changes in subcellular localization of β-catenin and β-catenin-associated cell adhesion molecules in HCC (Ihara et al., 1996). Studies have shown that 50-70% of liver tumors have increased levels of β-catenin in the cytoplasm and in the nucleus (Wong et al., 2001). Such accumulation can potentially provide tumor cells a growth advantage by promoting proliferation and inhibiting differentiation. A transgenic mouse model overexpressing β-catenin developed severe hepatomegaly (Giles et al., 2003). There are also studies associating β-catenin mutations or activation with worsened HCC outcome such as larger tumor size and increased vascular invasion (Behari, 2010).

Accumulation and stabilization of β-catenin could be a direct result from point mutations or deletions in the β-catenin gene, which is found in 12-26% of HCC (Giles et al., 2003). Such dominant gain-of-function mutations usually occur at the N-terminal phosphorylation sites on β-catenin, including the sites phosphorylated by GSK3β that regulate β-catenin degradation (Takigawa and Brown, 2008). Mutations at these positions disrupt recognition by GSK3β resulting in more stable β-catenin protein. Besides gain of function mutations in positive modulators of Wnt signaling such as β-catenin, the Wnt pathway can also be activated from loss-of function mutations in negative modulators such as Axin and APC (Takigawa and Brown, 2008). However, frequencies of β-catenin accumulation in HCC determined by immunostaining are much higher than the known incidence of Wnt pathway mutations. Thus, other causes of β-catenin accumulation may also exist. Epigenetic changes could be a contributing factor, which leads to higher gene expression without any mutations in the gene (Takigawa and Brown, 2008).

Although there is little doubt about the strong correlation between aberrant Wnt signaling and HCC, the precise role of activated Wnt pathway in the pathogenesis of liver tumor is less well understood. It has been shown that pharmacologic inhibition of β-catenin decreases survival of hepatoma cells (Behari et al., 2007). Inactivation of β-catenin suppressor APC led to spontaneous development of HCC in a mice model, suggesting the direct contribution from activated Wnt signaling to hepatocarcinogenesis (Colnot et al., 2004). However mice overexpressing a gain-of-function β-catenin mutant (exon 3 deletion) only showed increased susceptibility to developing HCC after exposure to carcinogen diethylnitrosamine (DEN), instead of developing spontaneous liver tumor (Harada et al., 2002). These results suggest that the role of the Wnt pathway in the development of liver cancer is highly context-dependent and involves cross-talk with other pathways. Nonetheless, components of the Wnt pathway may represent potential therapeutic intervention points for treating HCC.

Several approaches could be envisioned to target Wnt pathways. Extracellularly, it is possible to design molecules to disrupt Wnt ligand-receptor complexes, preventing initiation of the signaling events. For this approach, Wnt ligands, extracellular Wnt regulators such as DKKs, SFRPs proteins, and members of the receptor complexes can all be targeted. These proteins themselves or variants could be directly considered as candidates for drug developments. Alternatively, antibodies or other modalities that could block ligand receptor interactions may also be explored for therapeutic development. Intracellular components could also be targeted. Small molecule ligands toward kinases of the signaling pathway could also be screened that may regulate Wnt signaling. In addition, small molecule antagonists have been identified to interfere with the binding between β-catenin and TCF/LEF proteins or its coactivator CEBP, blocking downstream gene activation (Dahmani et al., 2011). However, due to the inherent complexity of Wnt signaling in the liver, further research is needed to fully understand the implications of therapeutic inhibition of the pathway in HCC.

2.2 VEGF pathway

Angiogenesis is critical for cancer development. Tumor cells require oxygen and nutrients for survival and proliferation and they need to be located within 100 to 200 μm from blood vessels to obtain an adequate supply of oxygen (Carmeliet and Jain, 2000). Solid tumors smaller than 1 to 2 cubic millimeters are not vascularized (Hawkins, 1995). However, beyond the critical volume of 2 cubic millimeters, new blood vessels need to be recruited to supply oxygen and nutrients and to remove metabolic wastes (Hawkins, 1995). Neovascularization also facilitates the dissemination of cancer cells throughout the entire body eventually leading to metastasis formation.

HCC is a hypervascular tumor and many pro-angiogenic factors are over-expressed in HCC cells and in the surrounding microenvironment (Shen et al., 2010). Among them, VEGF receptor signaling is one of the most well studied. The major VEGF that mediates tumor angiogenesis is VEGF-A, and it has several splicing variants that can be produced simultaneously. The most predominant forms are VEGF-A_{121} and VEGF-A_{165}. Other members in the VEGF family include Placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D (Kaseb et al., 2009; Roskoski, 2007). VEGF signals through VEGF receptor (tyrosine kinase receptor) on the cell surface. There are three main subtypes of VEGF receptors,

numbered 1, 2 and 3 (Kaseb et al., 2009; Roskoski, 2007). They all have an extracellular region consisting of 7 immunoglobulin-like domains, a single transmembrane domain, and an intracellular portion containing a split tyrosine-kinase domain. VEGF ligand binding induces dimerization and autophosphorylation of VEGF receptors. Phosphorylated tyrosine residues in the receptor serve as a docking site for various signal transduction proteins that can eventually activate cellular processes involved in angiogenesis. For example, VEGFR-2 phosphorylation activates PLC-γ which in turn leads to protein kinase C activation (PKC). PKC can activate MAP kinase signaling and promote cell proliferation as well as increase vascular permeability through activation of endothelial nitric oxide synthase (Kaseb et al., 2009; Roskoski, 2007). VEGF also induce activation of Rho GTPase, which plays a crucial role during angiogenesis processes such as vascular permeability, extra cellular matrix degradation, cellular migration and invasion (van der Meel et al., 2011).

Among the three VEGF receptors, VEGFR-2 appears to mediate almost all of the known cellular responses to VEGFs. The activation of VEGFR-2 in endothelial cells results in their proliferation, migration, and increased survival and promotes vascular permeability (Kaseb et al., 2009; Roskoski, 2007). The function of VEGFR-1 is less known. Although it has higher affinity for VEGF than VEFGR-2, it has weak tyrosine kinase phosphorylation activity following ligand stimulation. Activation of VEGFR-1 has no direct proliferative or cytoskeletal effects. It is possibly involved in modulating VEGFR-2 activity. VEGF-C and VEGF-D, but not VEGF-A, are ligands for a third receptor (VEGFR-3), which is important for lymphangiogenesis (Kaseb et al., 2009; Roskoski, 2007).

The VEGF pathway is clearly important for HCC pathogenesis. Expression of VEGF mRNA in liver tumors was found in a majority of HCC patients. And the expression of VEGF steadily increases with the progression of the hepatocarcinogenic process from a normal liver, to a dysplastic nodule, to HCC (Yamaguchi et al., 1998). The levels of VEGF mRNA expression in tumors with tumorous emboli and in poor-encapsulated tumors were higher than those without tumorous emboli and in well-encapsulated tumors. The principle route of HCC dissemination and metastasis is through the portal vein in the liver and VEGF mRNA level correlated well with portal vein tumor thrombus (PVTT) formation of HCC, suggesting VEGF may play an important role in HCC invasion and metastasis (Zhou et al., 2000). Immunohistochemical staining also detected very high VEGF expression in well-differentiated HCC as well as areas surrounding the HCC tissues, where inflammatory cell filtration was apparent. High serum VEGF levels have been shown to correlate with poor response to chemotherapy and poor survival among HCC patients. Increased preoperative serum VEGF may also predict high incidence of tumor recurrence after surgical resection (Whittaker et al., 2010). Furthermore, increased VEGF expression has also been detected in cirrhotic and dysplastic livers, which often lead to liver cancer (El-Assal et al., 1998).

The most direct evidence supporting the role of the VEGF signaling pathway in HCC came from recent progress in molecular targeted therapy inhibiting this pathway. Bevacizumab, an anti–VEGF monoclonal antibody was tested in patients with unresectable HCC and resulted in significant disease-stabilizing effect (Siegel et al., 2008). Clinical effects were assessed by tumor regression and progression-free survival (PFS). Of the 46 patients in the study, 13% had objective response and 65% were progression free at 6 months. The mean PFS time was 6.9 months and median overall survival time is 12.4 months. Overall survival rates were 53% at 1 year, 28% at 2 years and 23% at 3 years. Treatment was associated with

significant reductions in tumor arterial enhancement and circulating VEGF-A level. Besides VEGF antibody, small molecule VEGF inhibitors have also been developed and tested in clinic. Sorafenib, an inhibitor targeting the VEGF pathway has been shown to prolong overall survival in patient with advanced HCC (Llovet et al., 2008). In a randomized, placebo-controlled phase III trial, sorafenib prolonged median survival time of advanced HCC patients by 2.8 months, from 7.9 months in the placebo group to 10.7 months in the sorafenib treatment group. Time to radiologic progression was delayed by 2.7 months, from 2.8 months to 5.5 months. This result is quite significant since no effective systemic therapy ever existed for patients with advanced hepatocellular carcinoma before the sorafenib trial. There are several other VEGF small molecule inhibitors that are currently being tested in the clinic for HCC treatment including, sunitinib, vatalanib, cediranib, brivanib, and linifanib (Shen et al., 2010; Whittaker et al., 2010). Studies have also been carried out to assess the benefit of the combined therapy using those compounds.

2.3 FGF pathway

Supported by various mouse genetic models as well as human genetic studies, aberrant FGF/FGFR signaling is clearly associated with tumorigenesis (Beenken and Mohammadi, 2009; Knights and Cook, 2010; Krejci et al., 2009; Turner and Grose, 2010). Signaling is activated upon FGF ligands binding to the FGF receptors on the cell surface. There are more than 20 different FGFs in the FGF family, making it the largest family of growth factors. Fibroblast growth factor receptors consist of an extracellular ligand binding domain, a single transmembrane domain, and an intracellular domain with tyrosine kinase activity. The extracellular domain of the FGF receptor is composed of three immunoglobin-like domains. Alternative splicing of four FGF receptors genes (FGFR1-4) results in over 48 different isoforms of FGFRs, and they have different affinity towards different FGF ligands and have distinct expression patterns (Ornitz and Itoh, 2001; Ornitz et al., 1996). FGFRs are tyrosine kinase receptors that upon ligand binding induce dimerization and kinase activation in the presence of the co-factor, heparan sulfate (Plotnikov et al., 1999). Phosphorylation of the tyrosine residues on the receptor provides docking sites for downstream adaptor proteins, which can couple to the activation of different intracellular signaling pathways. One of the key adaptor proteins of FGF receptors is FGF substrate 2 (FRS2) which can be phosphorylated by FGFR receptors and recruit more adaptor proteins such as son of sevenless (SOS) and growth factor receptor-bound 2 (GRB2) to activate RAS GTPase (Eswarakumar et al., 2005). RAS GTPase promotes several downstream signaling, such as Wnt, MAPK, and PI3K/Akt pathways (Knights and Cook, 2010). These FGF downstream signaling pathways have all been implicated in several aspects of tumorigenesis, such as proliferation, survival, cell migration and invasion, as well as angiogenesis (Balmanno and Cook, 2009; Dailey et al., 2005; Presta et al., 2005; Xian et al., 2005). In HCC, it has been shown that plasma FGF2 level was significantly increased (Hsu et al., 1997) and overexpression of FGFR1 in hepatocytes accelerated the growth of HCC chemically induced by DEN in a mouse model (Huang et al., 2006).

Recently, attention has focused on a unique FGF family member, FGF19, and its involvement in the development of HCC (Nicholes et al., 2002; Wu and Li, 2009). FGF19 belongs to a unique FGF subfamily that has weakened affinity towards heparan sulfate. The reduced affinity liberates FGF19 from tissues where it is expressed, allowing it to act as an endocrine hormone. Furthermore, FGF19 requires βKlotho as a co-receptor in activating FGF

receptors. In the presence of βKlotho, FGF19 is able to activate similar downstream signaling through FGF receptors (Kurosu et al., 2007; Wu et al., 2007).

First evidence connecting FGF19 with HCC came from a transgenic FGF19 mice model, where human FGF19 driven by myosin promoters was overexpressed from skeleton muscle, resulting in elevated serum FGF19 protein levels (Nicholes et al., 2002). HCC development was observed in these FGF19 transgenic mice at 8-10 months, while no tumors were observed in wild type control mice. Tumors occurred in different liver lobes and were either solitary or multifocal. Histological staining showed neoplastic cells invasion and replacement of normal hepatocytes (Nicholes et al., 2002). Hepatocytes are normally mitotically quiescent in the liver. However, in case of HCC, hepatocellular proliferation is a prerequisite for transformation. In vivo 5-bromo-2'-deoxyuridine (BrdU) labeling was performed to assess the proliferation in FGF19 transgenic mice. BrdU-labeling index of hepatocytes was eight fold higher in the transgenic mice than age-matched wild type mice at 2 to 4 months of age (Nicholes et al., 2002). Furthermore, recombinant FGF19 protein also induced a significant higher BrdU-labeling index after being injected into normal mice (Nicholes et al., 2002; Wu et al., 2010a; Wu et al., 2010b). These results strongly support the notion that FGF19 can induce hepatocellular proliferation which eventually leads to development of HCC.

FGF19 is able to activate the Wnt pathway in hepatocytes, and 44% of neoplastic hepatocytes in FGF19 transgenic mice have nuclear staining for β-catenin (Nicholes et al., 2002; Pai et al., 2008). Since aberrant Wnt signaling correlates strongly with HCC tumorigenesis, this may be one mechanism whereby FGF19 induces liver tumor formation. To test the hypothesis of whether FGF19 could be a valid target in HCC treatment, a monoclonal anti-FGF19 antibody was generated and tested in tumor inhibition. Anti-FGF19 antibody or control antibody were used to treat DEN accelerated HCC formation in FGF19 transgenic mice (Desnoyers et al., 2008). After six months of antibody treatment, all the animals treated with control antibody developed multifocal, large HCCs throughout the liver lobes while almost none of the mice treated with FGF19 antibody had liver tumors. The same anti-FGF19 antibody was also tested in xenograft mice models (Desnoyers et al., 2008). Mice xenografted with colon cancer cell lines HCT116 and Colo201 were injected with anti-FGF19 antibody twice weekly. At day 35, FGF19 antibody suppressed tumor growth by 57% compared to the control in HCT116 group and 64% growth inhibition was achieved in Colo201 animals treated with the antibody. These data suggest that targeting FGF19 could be a valid strategy for HCC treatment.

The main candidate FGF receptor mediating FGF19 induced tumorigensis in liver is believed to be FGFR4, which is the predominant FGF receptor expressed in the liver. Strong FGFR4 mRNA was detected in hepatocytes adjacent to central vein by in situ hybridization, and the hepatic dysplasia foci and BrdU labeling from FGF19 transgenic livers are also located around central vein. In addition, activation of FGFR4 alone was sufficient to induce hepatocyte proliferation (Wu et al., 2010a; Wu et al., 2010b) and FGF19 induced hepatocytes proliferation was not observed in FGFR4 knockout mice, confirming the role played by FGFR4 in hepatocarcinogenesis (Wu et al., 2011).

FGFR4 has been implicated in HCC in many literature reports (Yang et al., 2011). FGFR4 is frequently overexpressed in patients with hepatocellular carcinoma (Yang et al., 2011). siRNA against FGFR4 in liver cancer lines HuH7 is able to suppress α-fetoprotein

production (Yang et al., 2011). However, there have also been reports showing genetic deletion of FGFR4 in mice results in faster progression of DEN-accelerated hepatocellular carcinoma, suggesting that FGFR4 suppresses hepatoma proliferation (Huang et al., 2009). The contribution of FGFR4 in HCC progression requires further clarification.

2.4 MAPK pathway

Mitogen-activated protein kinase (MAPK) is serine-threonine kinase that is involved in a variety of cellular activities. There are three members in the mammalian MAPK family, extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 (Kim and Choi, 2010; Min et al., 2011). Among them, ERK signaling pathway is the most studied for its involvement in promoting cell proliferation, migration, survival, and its association with tumorigenesis and tumor progression (Gollob et al., 2006). Recent data also implicate JNK and p38 as playing important roles during HCC development (Min et al., 2011).

The ERK pathway is ubiquitous and can be activated by various receptors, particularly receptor tyrosine kinases (RTKs). Upon ligand binding, the RTKs dimerize which leads to activation of the intracellular tyrosine kinase domain. Activated kinase results in receptor phosphorylation on tyrosine residues, that then serves as docking sites for adaptor proteins such as GRB2 and SOS (Schulze et al., 2005; Zarich et al., 2006). Upon docking to the receptors, the GRB2 and SOS activate the small GTPase RAS (HRAS, NRAS and KRAS) which in turn will activate the serine/threonine kinase RAF. RAF is a MAPK kinase kinase (MAP3K), and it has three isoforms, ARAF, BRAF and CRAF. Activated RAF will phosphorylate and activate MEK, a MAPK kinase (MAP2K) and MEK is the kinase for ERK (ERK1 and ERK2) (Avruch et al., 2001; Malumbres and Barbacid, 2003).

There are more than 100 substrates downstream of ERK, and many of them are transcription factors. The altered levels and activities of the transcription factors by ERK activation can lead to altered expression levels of genes that are important for cell cycle progression (Davis, 1995). For example, ERK can phosphorylate and activate C-myc, a transcription factor that regulates the expression of many target genes involved in cell growth and proliferation. C-Myc is a strong proto-oncogene and can be found unregulated in many types of cancers (Penn et al., 1990). ERK also directly phosphorylates kinase substrates such as myosin light chain kinase, calpain, and focal adhesion kinase, which promotes cell migration (Huang et al., 2004). Furthermore, the ERK pathway can regulate proteins involved in apoptotic pathway such as BIM and MCL1, promoting survival of cancer cells (Balmanno and Cook, 2009).

Given the contribution of ERK signaling towards cell proliferation, migration and survival, it is not surprising to see constitutive activation of the ERK pathway in many tumors. In fact, genes along the ERK pathway, such as HRAS, KRAS, and CRAF, are often upregulated in HCC. One study has shown that CRAF is overexpresed among all 30 HCC tissue samples tested (Hwang et al., 2004). Immunostaining also showed around 7 fold increase of MEK phosphorylation in HCC tissues compared to surrounding benign liver tissues (Huynh et al., 2003). Other studies also found that phosphorylated ERK level is higher in HCC tissues and ERK activation is associated with aggressive tumor behavior (Schmitz et al., 2008). In addition, negative regulator proteins of MAPK/ERK pathway such as Sprouty and DUSP1 are down-regulated in HCC tumors (Calvisi et al., 2008) (Yoshida et al., 2006). Sustained

activation of ERK signaling can also occur due to point mutations in the RAS gene, which leads to constitutive CRAF activation (Downward, 2003; Whittaker et al., 2010). Mutation in the RAS gene has been reported in 10-30% of HCC tumors (Whittaker et al., 2010). The involvement of the ERK pathway in HCC is further confirmed by preclinical studies using the MEK inhibitor, AZD6244, which blocks proliferation and promotes apoptosis in primary HCC cells (Huynh et al., 2007a; Huynh et al., 2007b). AZD6244 also suppresses tumor growth in HCC xenograft model in a dose-dependent manner. And tumor growth inhibition after AZD6244 treatment correlates with inactivation of ERK, up-regulation of apoptotic genes such as caspase-3 and 7 and down-regulation of cell cycle regulators such as cyclin D1. It has also been shown that AZD6244 can induce a synergistic effect in tumor suppression when combined with chemotherapeutic agent doxorubicin (Huynh et al., 2007a; Huynh et al., 2007b).

JNK is another major MAPK signaling pathway. It can be activated by two MAPK kinases, MKK4 and MKK7, and its downstream substrates include c-Jun (Keshet and Seger, 2010). JNK signaling can be activated by various cytokines and environmental factors. It has been demonstrated that JNK1 and JNK2 regulate stress-induced apoptosis, and increased JNK activity has also been shown to enhance proliferation of mouse embryonic fibroblasts (MEFs) (Das et al., 2011). There is strong correlation between activated JNK signaling and HCC. For example, one study has shown that JNK1 is over-activated in 17 out of 31 samples (55%) from Chinese HCC patients (Chang et al., 2009). The activation in JNK1 is associated with increased tumor size and a lack of encapsulation of the tumors. In addition, JNK1 activation also associates with increased histone H3 lysines 4 and 9 tri-methylation, which leads to up-regulation of genes promoting cell growth (Chang et al., 2009).

Direct evidence demonstrating the role of JNK pathway in HCC development comes from studies in mouse models. JNK1 knockout mice had a significant reduction in liver tumorigenesis chemically induced by DEN and hepatocyte proliferation also decreased in those animals (Hui et al., 2008). It was proposed that mice lacking JNK1 have increased expression of p21, a cell cycle inhibitor. Blocking JNK activity using pharmacological inhibitor D-JNKI1 reduced growth of both xenografted human HCC cells and DEN-induced mouse liver cancers, further supporting the role played by JNK pathway in HCC development (Hui et al., 2008).

The activation of p38 is induced by MKK3, 4, and 6, as well as autophosphorylation. Its substrates include transcription factors such as p53 and protein kinases such as MK2 and MK5 (Min et al., 2011). Interestingly, unlike ERK and JNK pathways, p38 seems to play a suppressive role in HCC (Min et al., 2011). It was shown that human embryonic fibroblasts displayed enhanced proliferation upon treatment with a p38 inhibitor (SB203580) (Wang et al., 2002). p38 negatively regulates proliferation partly through suppression of the JNK pathway, which is known to promote cell proliferation. Depending upon the cellular context, p38 can enhance the protein level of JNK phosphatase and repress the activities of JNK kinases (Wagner and Nebreda, 2009). In p38 deficient fetal liver cells and liver tumor cells, the JNK pathway activity was found to be increased. Direct evidence supporting the suppressive role of p38 in HCC came from a study using mice with liver-specific deletion of p38, where enhanced hepatocyte proliferation and tumor development were observed during liver carcinogenesis (Hui et al., 2007). After DEN treatment, p38 deficient mice clearly developed more tumors in the liver and had larger average tumor size compared

with control mice. Moreover, inactivation of JNK pathway with Δ-JNKI1 suppressed the hyperproliferation in p38-deficient animals (Hui et al., 2007).

In summary, numerous data have clearly demonstrated the deep involvement of MAPK signaling pathways during liver carcinogenesis. Identifying pharmacological intervention points along these pathways could be considered as a very promising strategy for combating HCC.

2.5 PI3k/AKT/mTOR pathway

Phosphoinositide 3 kinase (PI3K) is an intracellular signal transducer enzyme that can phosphorylate the hydroxyl group of phosphatidylinositol (Vanhaesebroeck and Waterfield, 1999). It belongs to a large family of PI3K-related kinases (Kuruvilla and Schreiber, 1999). PI3K is comprised of a catalytic subunit and a regulatory subunit. The regulatory subunit p85 can interact with phosphotyrosines on activated RTKs that recruit the enzyme to the plasma membrane and activate the enzymatic activities (Paez and Sellers, 2003). PI3K produces the lipid second messenger phosphatidylinosoltriphosphate, which is absent in resting cells but can be acutely produced in response to activated PI3K (Vanhaesebroeck and Waterfield, 1999).

Akt is a serine-threonine kinase downstream of PI3K. It contains a pleckstrin homology (PH) domain in the N-terminus, a central catalytic kinase domain and a C-terminus regulatory domain (Paez and Sellers, 2003). The PH domain will bind to phosphatidylinosoltriphosphate with high affinity. Upon PI3K activation and phosphatidylinosoltriphosphate production, Akt is recruited to the plasma membrane through its PH domain together with another PH domain containing protein, phosphoinositide dependent kinase 1 (PDK1). PDK1 then phosphorylate key residues in the kinase domain activation loop of Akt to activate Akt kinase activity (Paez and Sellers, 2003).

Activated Akt phosphorylates multiple protein substrates and regulates a variety of critical cellular activities (Paez and Sellers, 2003; Whittaker et al., 2010). Mammalian target of rapamycin (mTOR) is one of the most important downstream effectors of Akt. Akt phosphorylates the tuberous sclerosis complex (TSC1/TSC2) which activates the small G protein, Ras homolog enriched in brain (Rheb). Rheb, in its GTP-bound state, can activate mTOR. mTOR is a serine-threonine protein kinase that also belongs to PI3K-related kinase family. It is a large protein consists of tandem HEAT repeats, FAT (FRAP-ATM-TRRAP) and FATC (FAT C-terminus) domains, FKBP12-rapamycin binding domain (FRB), and C-terminus catalytic kinase domain that resembles the catalytic domain of PI3K (Wullschleger et al., 2006). For mTOR to activate its signaling cascade, it must form the ternary complex mTORC1 (mTOR Complex-1) or mTORC2 (mTOR Complex-2), but mTOR is the catalytic subunit of both of these two complexes. mTORC1 complex contains mTOR, RAPTOR (Regulatory Associated Protein of mTOR), mammalian LST8/G-protein β-subunit like protein (mLST8/GβL), PRAS40 and DEPTOR. mTORC2 complex consists of mTOR, rapamycin-insensitive companion of mTOR (Rictor), GβL, and mammalian stress-activated protein kinase interacting protein 1 (mSIN1) (Wullschleger et al., 2006; Paez and Sellers, 2003).

Another very important component of PI3K/Akt/mTOR pathway is PTEN (phosphatase and tensin homolog). It consists of a phosphatase domain which carries out the enzymatic function

and a C2 domain which binds the phospholipid membrane. PTEN dephosphorylates phosphatidylinosoltriphosphate and serves as a negative regulator of PI3K/AKT/mTOR pathway (Paez and Sellers, 2003).

mTOR controls several important cellular processes including regulation of protein translation. Abberent protein translation often leads to abnormal cell growth and tumorigenesis (Petroulakis et al., 2006; Wullschleger et al., 2006). mTOR enhances translation initiation via two major targets, the eIF4E binding proteins (4E-BPs) and the ribosomal protein S6 kinases (S6K1 and S6K2). Eukaryotic mRNAs contain a 'cap' structure, m7GpppN at the 5' end and can be specifically recognized by the initiation factor eIF4E, which associates with eIF4G and eIF4A to form eIF4F complex and initiate cap-dependent translation. 4E-BP binds to eIF4E and inhibits eIF4F complex formation. Upon phosphorylation of 4E-BP by mTOR, eIF4E is released to stimulate translation initiation. S6 kinase is activated by mTOR and phosphorylates 40S ribosomal protein S6, which leads to increased translation of a subset of mRNAs containing 5' tract of oligopyrimidine (TOP). 5' TOP mRNAs encode ribosomal proteins, elongation factors, the poly-A binding protein and other components of the translational machinery. Therefore, stimulation of the 5' TOP mRNA translation by S6 results in up-regulation of the overall cellular translation capacity (Petroulakis et al., 2006).

The PI3K/Akt/mTOR signaling pathway is known to be up-regulated in various carcinoma cell lines, as well as in human ovarian and breast carcinomas (Altomare et al., 2004) (McAuliffe et al., 2010). For HCC, one study has shown overexpression of phospho-mTOR in 15% of liver tumors. Phospho-mTOR also positivity correlated with increased expression of total S6 kinase, which was found in 45% of the cases (Sahin et al., 2004). Elevated Akt phosphorylation was also found in 23% of HCC and implicated early HCC recurrence and poor prognosis (Boyault et al., 2007). There is also a high frequency (35.6%) of somatic PI3K mutations in HCC specimens (Lee et al., 2005). PTEN, the negative component of the PI3K/Akt/mTOR, is mutated in 5% of HCC and its expression is reduced in half of all HCC tumors, leading to the over activation of the pathway (Whittaker et al., 2010). In HCC patients, reduced PTEN expression has been associated with advanced tumor stage, high recurrences rate and poor survival outcome, suggesting inactivation of PTEN is involved in the pathogenesis of HCC (Hu et al., 2003). A study using hepatocyte-specific PTEN deficient mice further supported such a connection, by demonstrating that at 80 weeks of age, 66% of PTEN-deficient mice developed HCC (Horie et al., 2004).

Given the strong association between aberrant PI3K/Akt/mTOR signaling and HCC, pharmacological inhibition of this pathway could be a viable HCC treatment strategy. The mTOR inhibitor everolimus has been shown to decrease the growth of HCC cell line (Villanueva et al., 2008). Everolimus also induced a significant delay of tumor growth in a HCC xenograft mice model (Villanueva et al., 2008). In a separate study, the mTOR inhibitor sirolimus was tested in a rat HCC model and the treatment resulted in significantly longer survival time, smaller tumor size, and fewer extrahepatic metastases in those animals (Semela et al., 2007). Sirolimus has also been tested in human HCC patients, and it induced a partial response in 5% of the patients and tumor stabilization for at least 3 months in 24% of the patients. Another study has shown that 33% of patients partially response to sirolimus treatment (Rizell et al., 2008; Semela et al., 2007). Several new clinical trials are currently testing mTOR inhibitors and its combination with other therapies among HCC patients.

2.6 Other miscellaneous pathways

The EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase which is activated by ligands including epidermal growth factor (EGF) and transforming growth factor α (TGF-α) (Herbst, 2004). Upon activation, EGFR forms a dimer and autophosphorylates the tyrosine residues in its intracellular cytoplasmic domain, which in turn leads to initiation of many downstream signal transduction cascades (Herbst, 2004). The EGFR signaling pathway is one of the most important pathways that regulate growth, survival, proliferation, and differentiation in mammalian cells. Numerous studies have shown that aberrant EGFR signaling plays a vital role in tumor angiogenesis and proliferation and agents that specifically block this pathway showed efficacy in several types of solid tumors (Zhang et al., 2007). For HCC, EGFR overexpression was detected among 40–70% of the tumors and TGF-α level was also elevated in pre-neoplastic HCC (Feitelson et al., 2004). Thus, targeting EGFR may also show beneficial effect for HCC patients. EGFR blocking agents include both small molecule tyrosine kinase inhibitors and monoclonal antibodies targeting the receptor. Erlotinib is a low molecular weight inhibitor of EGFR kinase (Thomas et al., 2007). It is able to suppress the growth of HCC cell lines *in vitro*. Furthermore, during a Phase 2 clinical trial, 17 out of 40 patients with unresectable HCC achieved stable disease at 16 weeks of erlotinib treatment. Progression-free survival at 16 weeks was 43% and median overall survival was 43 weeks, longer than the historical controls. Monoclonal antibody cetuximab which targets the extracelluar domain of EGFR has also been tested in HCC (Zhu et al., 2007). Although cetuximab was able to inhibit cell growth and induce apoptosis in some HCC cell lines, the results from clinical trials testing its efficacy among HCC patients have been inconsistent. Currently, several more anti-EGFR pathway compounds are being tested in clinic, in some cases in combination with other therapeutic methods. The results from those trials might further clarify the benefit of the EGFR blocking agents for HCC.

Deregulation of the insulin-like growth factor (IGF) pathway has also been implicated in the development of HCC (Scharf et al., 2001). IGF-1 and 2 bind to IGF receptor IGF-1R and activate downstream signaling (Alexia et al., 2004). IGF signaling pathway regulates cell proliferation, motility and apoptosis. Pronounced alterations in the expression of components of the IGF pathway have been reported during hepatocarcinogenesis (Whittaker et al., 2010). IGF-2 is overexpressed in 16-40% of HCC and around 30% of HCCs overexpress IFG-1R (Cariani et al., 1988). Neutralizing IGF-2 has been shown to reduce cell proliferation and increase apoptosis in HCC cell lines (Lund et al., 2004). Furthermore, a monoclonal antibody that selectively inhibits IGF-1R was not only able to decrease viability and proliferation of liver cancer cells *in vitro* but also delay tumor growth and prolong survival in HCC xenograft mice model (Tovar et al., 2010). Several small molecules and monoclonal antibodies targeting IGF-1R are now under early clinical development.

In HCC, the transforming growth factor-beta (TGF-β) pathway regulates several steps in tumor progression, including angiogenesis, production of the extracellular matrix and immune suppression (Giannelli et al., 2011). It is also involved in initiating signaling cascade which promotes liver fibrosis, cirrhosis and subsequent progression to HCC (Giannelli et al., 2011). Increased levels of TGF-β in HCC patients' sera and urine are associated with disease progression (Yasmin Anum et al., 2009; Tsai et al., 1997). Specific small molecule inhibitors targeting TGF-β type I receptor (TGF-βRI) kinase LY2109761 reduce migration of HCC cells

and blocks invasion of HCC cells into the tissue microenvironment and blood vessels. LY2109761 also is effective in blocking tumor growth in a HCC xenograft chick embryo model and this antitumor activity was associated with anti-angiogenic effect (Mazzocca et al., 2009).

Hepatocyte growth factor (HGF) is a cytokine secreted by mesenchymal cells. It can stimulate mitogenesis, cell motility and has been implicated in tumor invasion. HGF is secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and 34-kDa beta-chain (Matsumoto and Nakamura, 1996). Active HGF is a herterodimer between alpha-chain and beta-chain linked by disulfide bond. HGF is homologous to the plasminogen subfamily of S1 peptidases but has no detectable protease activity. The proto oncogene c-Met is a receptor for HGF that is a heterodimer composed of a 50-kDa alpha-chain and a membrane spanning 145-kDa beta-chain with tyrosine kinase activity (Matsumoto and Nakamura, 1996). Activation of c-Met by HGF has been shown to activate MAPK, PI3K and Wnt signaling (Whittaker et al., 2010; Apte et al., 2006). Overexpression of c-Met was noted in 20-48% HCC tissues compared to surrounding non-cancerous liver tissues, and the overexpression levels correlated with worsening behavior of HCC and decreased 5-year survival in HCC patients (Whittaker et al., 2010; Ueki et al., 1997). A preclinical study reported that inhibition of c-Met by small molecule inhibitor SU11274 decreased HCC cell growth (Inagaki et al., 2011).

3. Conclusion

Many critical signaling pathways have been indicated in HCC development and a considerable amount of crosstalk and redundancy among those pathways have been observed. Various strategies targeting these pathways have been explored and have shown varying degrees of success in the clinic. Additional novel strategies are being investigated and raise the hope that more effective therapies may be on the horizon. However, HCC is a complex disease and aberrations in signaling pathways can vary from patient to patient. In addition, defects in multiple pathways may also in combination contribute to the pathogenesis. Uncovering the specific signaling pathways defects in the individual patient and the potential use of combination therapies may be critical for generating effective treatment outcomes in the future.

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5. References

Alexia, C., Fallot, G., Lasfer, M., Schweizer-Groyer, G., and Groyer, A. (2004). An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. Biochem Pharmacol *68*, 1003-1015.

Altomare, D.A., Wang, H.Q., Skele, K.L., De Rienzo, A., Klein-Szanto, A.J., Godwin, A.K., and Testa, J.R. (2004). AKT and mTOR phosphorylation is frequently detected in

ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. Oncogene *23*, 5853-5857.

Alves, R.C., Alves, D., Guz, B., Matos, C., Viana, M., Harriz, M., Terrabuio, D., Kondo, M., Gampel, O., and Polletti, P. (2011). Advanced hepatocellular carcinoma. Review of targeted molecular drugs. Ann Hepatol *10*, 21-27.

Ansari, J., Palmer, D.H., Rea, D.W., and Hussain, S.A. (2009). Role of tyrosine kinase inhibitors in lung cancer. Anticancer Agents Med Chem *9*, 569-575.

Anzola, M. (2004). Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat *11*, 383-393.

Apte, U., Zeng, G., Muller, P., Tan, X., Micsenyi, A., Cieply, B., Dai, C., Liu, Y., Kaestner, K.H., and Monga, S.P. (2006). Activation of Wnt/beta-catenin pathway during hepatocyte growth factor-induced hepatomegaly in mice. Hepatology *44*, 992-1002.

Avruch, J., Khokhlatchev, A., Kyriakis, J.M., Luo, Z., Tzivion, G., Vavvas, D., and Zhang, X.F. (2001). Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. Recent Prog Horm Res *56*, 127-155.

Balmanno, K., and Cook, S.J. (2009). Tumour cell survival signalling by the ERK1/2 pathway. Cell Death Differ *16*, 368-377.

Beenken, A., and Mohammadi, M. (2009). The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov *8*, 235-253.

Behari, J. (2010). The Wnt/beta-catenin signaling pathway in liver biology and disease. Expert Rev Gastroenterol Hepatol *4*, 745-756.

Behari, J., Zeng, G., Otruba, W., Thompson, M.D., Muller, P., Micsenyi, A., Sekhon, S.S., Leoni, L., and Monga, S.P. (2007). R-Etodolac decreases beta-catenin levels along with survival and proliferation of hepatoma cells. J Hepatol *46*, 849-857.

Bosch, F.X., Ribes, J., Diaz, M., and Cleries, R. (2004). Primary liver cancer: worldwide incidence and trends. Gastroenterology *127*, S5-S16.

Boyault, S., Rickman, D.S., de Reynies, A., Balabaud, C., Rebouissou, S., Jeannot, E., Herault, A., Saric, J., Belghiti, J., Franco, D., *et al.* (2007). Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology *45*, 42-52.

Calvisi, D.F., Pinna, F., Meloni, F., Ladu, S., Pellegrino, R., Sini, M., Daino, L., Simile, M.M., De Miglio, M.R., Virdis, P., *et al.* (2008). Dual-specificity phosphatase 1 ubiquitination in extracellular signal-regulated kinase-mediated control of growth in human hepatocellular carcinoma. Cancer Res *68*, 4192-4200.

Cariani, E., Lasserre, C., Seurin, D., Hamelin, B., Kemeny, F., Franco, D., Czech, M.P., Ullrich, A., and Brechot, C. (1988). Differential expression of insulin-like growth factor II mRNA in human primary liver cancers, benign liver tumors, and liver cirrhosis. Cancer Res *48*, 6844-6849.

Carmeliet, P., and Jain, R.K. (2000). Angiogenesis in cancer and other diseases. Nature *407*, 249-257.

Chang, Q., Zhang, Y., Beezhold, K.J., Bhatia, D., Zhao, H., Chen, J., Castranova, V., Shi, X., and Chen, F. (2009). Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. J Hepatol *50*, 323-333.

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. Cell *127*, 469-480.

Colnot, S., Decaens, T., Niwa-Kawakita, M., Godard, C., Hamard, G., Kahn, A., Giovannini, M., and Perret, C. (2004). Liver-targeted disruption of Apc in mice activates beta-catenin signaling and leads to hepatocellular carcinomas. Proc Natl Acad Sci U S A *101*, 17216-17221.

Dahmani, R., Just, P.A., and Perret, C. (2011). The Wnt/beta-catenin pathway as a therapeutic target in human hepatocellular carcinoma. Clin Res Hepatol Gastroenterol.

Dailey, L., Ambrosetti, D., Mansukhani, A., and Basilico, C. (2005). Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev *16*, 233-247.

Dale, T.C. (1998). Signal transduction by the Wnt family of ligands. Biochem J *329 (Pt 2)*, 209-223.

Das, M., Garlick, D.S., Greiner, D.L., and Davis, R.J. (2011). The role of JNK in the development of hepatocellular carcinoma. Genes & development *25*, 634-645.

Davis, R.J. (1995). Transcriptional regulation by MAP kinases. Mol Reprod Dev *42*, 459-467.

Desnoyers, L.R., Pai, R., Ferrando, R.E., Hotzel, K., Le, T., Ross, J., Carano, R., D'Souza, A., Qing, J., Mohtashemi, I., *et al.* (2008). Targeting FGF19 inhibits tumor growth in colon cancer xenograft and FGF19 transgenic hepatocellular carcinoma models. Oncogene *27*, 85-97.

Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer *3*, 11-22.

El-Assal, O.N., Yamanoi, A., Soda, Y., Yamaguchi, M., Igarashi, M., Yamamoto, A., Nabika, T., and Nagasue, N. (1998). Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. Hepatology *27*, 1554-1562.

El-Serag, H.B., Hampel, H., and Javadi, F. (2006). The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol *4*, 369-380.

Eswarakumar, V.P., Lax, I., and Schlessinger, J. (2005). Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev *16*, 139-149.

Fedi, P., Bafico, A., Nieto Soria, A., Burgess, W.H., Miki, T., Bottaro, D.P., Kraus, M.H., and Aaronson, S.A. (1999). Isolation and biochemical characterization of the human Dkk-1 homologue, a novel inhibitor of mammalian Wnt signaling. J Biol Chem *274*, 19465-19472.

Feitelson, M.A., Pan, J., and Lian, Z. (2004). Early molecular and genetic determinants of primary liver malignancy. Surg Clin North Am *84*, 339-354.

Finch, P.W., He, X., Kelley, M.J., Uren, A., Schaudies, R.P., Popescu, N.C., Rudikoff, S., Aaronson, S.A., Varmus, H.E., and Rubin, J.S. (1997). Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. Proc Natl Acad Sci U S A *94*, 6770-6775.

Fujino, T., Asaba, H., Kang, M.J., Ikeda, Y., Sone, H., Takada, S., Kim, D.H., Ioka, R.X., Ono, M., Tomoyori, H., *et al.* (2003). Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion. Proc Natl Acad Sci U S A *100*, 229-234.

Giannelli, G., Mazzocca, A., Fransvea, E., Lahn, M., and Antonaci, S. (2011). Inhibiting TGF-beta signaling in hepatocellular carcinoma. Biochim Biophys Acta *1815*, 214-223.

Giles, R.H., van Es, J.H., and Clevers, H. (2003). Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta *1653*, 1-24.

Gollob, J.A., Wilhelm, S., Carter, C., and Kelley, S.L. (2006). Role of Raf kinase in cancer: therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. Semin Oncol *33*, 392-406.

Gordon, M.D., and Nusse, R. (2006). Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem *281*, 22429-22433.

Harada, N., Miyoshi, H., Murai, N., Oshima, H., Tamai, Y., Oshima, M., and Taketo, M.M. (2002). Lack of tumorigenesis in the mouse liver after adenovirus-mediated expression of a dominant stable mutant of beta-catenin. Cancer Res *62*, 1971-1977.

Hassan, M.M., Curley, S.A., Li, D., Kaseb, A., Davila, M., Abdalla, E.K., Javle, M., Moghazy, D.M., Lozano, R.D., Abbruzzese, J.L., *et al.* (2010). Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. Cancer *116*, 1938-1946.

Hawkins, M.J. (1995). Clinical trials of antiangiogenic agents. Curr Opin Oncol *7*, 90-93.

Herbst, R.S. (2004). Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys *59*, 21-26.

Horie, Y., Suzuki, A., Kataoka, E., Sasaki, T., Hamada, K., Sasaki, J., Mizuno, K., Hasegawa, G., Kishimoto, H., Iizuka, M., *et al.* (2004). Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. The Journal of clinical investigation *113*, 1774-1783.

Hsu, P.I., Chow, N.H., Lai, K.H., Yang, H.B., Chan, S.H., Lin, X.Z., Cheng, J.S., Huang, J.S., Ger, L.P., Huang, S.M., *et al.* (1997). Implications of serum basic fibroblast growth factor levels in chronic liver diseases and hepatocellular carcinoma. Anticancer research *17*, 2803-2809.

Hu, T.H., Huang, C.C., Lin, P.R., Chang, H.W., Ger, L.P., Lin, Y.W., Changchien, C.S., Lee, C.M., and Tai, M.H. (2003). Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. Cancer *97*, 1929-1940.

Huang, C., Jacobson, K., and Schaller, M.D. (2004). MAP kinases and cell migration. J Cell Sci *117*, 4619-4628.

Huang, X., Yang, C., Jin, C., Luo, Y., Wang, F., and McKeehan, W.L. (2009). Resident hepatocyte fibroblast growth factor receptor 4 limits hepatocarcinogenesis. Molecular carcinogenesis *48*, 553-562.

Huang, X., Yu, C., Jin, C., Kobayashi, M., Bowles, C.A., Wang, F., and McKeehan, W.L. (2006). Ectopic activity of fibroblast growth factor receptor 1 in hepatocytes accelerates hepatocarcinogenesis by driving proliferation and vascular endothelial growth factor-induced angiogenesis. Cancer Res *66*, 1481-1490.

Hui, L., Bakiri, L., Mairhorfer, A., Schweifer, N., Haslinger, C., Kenner, L., Komnenovic, V., Scheuch, H., Beug, H., and Wagner, E.F. (2007). p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. Nat Genet *39*, 741-749.

Hui, L., Zatloukal, K., Scheuch, H., Stepniak, E., and Wagner, E.F. (2008). Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-

dependent p21 downregulation. The Journal of clinical investigation *118*, 3943-3953.

Huynh, H., Chow, P.K., and Soo, K.C. (2007a). AZD6244 and doxorubicin induce growth suppression and apoptosis in mouse models of hepatocellular carcinoma. Mol Cancer Ther *6*, 2468-2476.

Huynh, H., Nguyen, T.T., Chow, K.H., Tan, P.H., Soo, K.C., and Tran, E. (2003). Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol *3*, 19.

Huynh, H., Soo, K.C., Chow, P.K., and Tran, E. (2007b). Targeted inhibition of the extracellular signal-regulated kinase kinase pathway with AZD6244 (ARRY-142886) in the treatment of hepatocellular carcinoma. Mol Cancer Ther *6*, 138-146.

Hwang, Y.H., Choi, J.Y., Kim, S., Chung, E.S., Kim, T., Koh, S.S., Lee, B., Bae, S.H., Kim, J., and Park, Y.M. (2004). Over-expression of c-raf-1 proto-oncogene in liver cirrhosis and hepatocellular carcinoma. Hepatol Res *29*, 113-121.

Ihara, A., Koizumi, H., Hashizume, R., and Uchikoshi, T. (1996). Expression of epithelial cadherin and alpha- and beta-catenins in nontumoral livers and hepatocellular carcinomas. Hepatology *23*, 1441-1447.

Inagaki, Y., Qi, F., Gao, J., Qu, X., Hasegawa, K., Sugawara, Y., Tang, W., and Kokudo, N. (2011). Effect of c-Met inhibitor SU11274 on hepatocellular carcinoma cell growth. Biosci Trends *5*, 52-56.

Kaseb, A.O., Hanbali, A., Cotant, M., Hassan, M.M., Wollner, I., and Philip, P.A. (2009). Vascular endothelial growth factor in the management of hepatocellular carcinoma: a review of literature. Cancer *115*, 4895-4906.

Keshet, Y., and Seger, R. (2010). The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. Methods Mol Biol *661*, 3-38.

Kim, E.K., and Choi, E.J. (2010). Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta *1802*, 396-405.

Knights, V., and Cook, S.J. (2010). De-regulated FGF receptors as therapeutic targets in cancer. Pharmacol Ther *125*, 105-117.

Krejci, P., Prochazkova, J., Bryja, V., Kozubik, A., and Wilcox, W.R. (2009). Molecular pathology of the fibroblast growth factor family. Hum Mutat *30*, 1245-1255.

Kurosu, H., Choi, M., Ogawa, Y., Dickson, A.S., Goetz, R., Eliseenkova, A.V., Mohammadi, M., Rosenblatt, K.P., Kliewer, S.A., and Kuro-o, M. (2007). Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem *282*, 26687-26695.

Kuruvilla, F.G., and Schreiber, S.L. (1999). The PIK-related kinases intercept conventional signaling pathways. Chem Biol *6*, R129-136.

Lee, J.W., Soung, Y.H., Kim, S.Y., Lee, H.W., Park, W.S., Nam, S.W., Kim, S.H., Lee, J.Y., Yoo, N.J., and Lee, S.H. (2005). PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene *24*, 1477-1480.

Llovet, J.M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J.F., de Oliveira, A.C., Santoro, A., Raoul, J.L., Forner, A., *et al.* (2008). Sorafenib in advanced hepatocellular carcinoma. N Engl J Med *359*, 378-390.

Lund, P., Schubert, D., Niketeghad, F., and Schirmacher, P. (2004). Autocrine inhibition of chemotherapy response in human liver tumor cells by insulin-like growth factor-II. Cancer Lett *206*, 85-96.

Malumbres, M., and Barbacid, M. (2003). RAS oncogenes: the first 30 years. Nat Rev Cancer *3*, 459-465.

Matsumoto, K., and Nakamura, T. (1996). Emerging multipotent aspects of hepatocyte growth factor. J Biochem *119*, 591-600.

Mazzocca, A., Fransvea, E., Lavezzari, G., Antonaci, S., and Giannelli, G. (2009). Inhibition of transforming growth factor beta receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. Hepatology *50*, 1140-1151.

McAuliffe, P.F., Meric-Bernstam, F., Mills, G.B., and Gonzalez-Angulo, A.M. (2010). Deciphering the role of PI3K/Akt/mTOR pathway in breast cancer biology and pathogenesis. Clin Breast Cancer *10 Suppl 3*, S59-65.

Min, L., He, B., and Hui, L. (2011). Mitogen-activated protein kinases in hepatocellular carcinoma development. Semin Cancer Biol *21*, 10-20.

Nelson, W.J., and Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. Science *303*, 1483-1487.

Nicholes, K., Guillet, S., Tomlinson, E., Hillan, K., Wright, B., Frantz, G.D., Pham, T.A., Dillard-Telm, L., Tsai, S.P., Stephan, J.P., *et al.* (2002). A mouse model of hepatocellular carcinoma: ectopic expression of fibroblast growth factor 19 in skeletal muscle of transgenic mice. The American journal of pathology *160*, 2295-2307.

Ornitz, D.M., and Itoh, N. (2001). Fibroblast growth factors. Genome biology *2*, REVIEWS3005.

Ornitz, D.M., Xu, J., Colvin, J.S., McEwen, D.G., MacArthur, C.A., Coulier, F., Gao, G., and Goldfarb, M. (1996). Receptor specificity of the fibroblast growth factor family. J Biol Chem *271*, 15292-15297.

Paez, J., and Sellers, W.R. (2003). PI3K/PTEN/AKT pathway. A critical mediator of oncogenic signaling. Cancer Treat Res *115*, 145-167.

Pai, R., Dunlap, D., Qing, J., Mohtashemi, I., Hotzel, K., and French, D.M. (2008). Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating beta-catenin signaling. Cancer Res *68*, 5086-5095.

Parkin, D.M., Bray, F., Ferlay, J., and Pisani, P. (2005). Global cancer statistics, 2002. CA Cancer J Clin *55*, 74-108.

Penn, L.J., Laufer, E.M., and Land, H. (1990). C-MYC: evidence for multiple regulatory functions. Semin Cancer Biol *1*, 69-80.

Petroulakis, E., Mamane, Y., Le Bacquer, O., Shahbazian, D., and Sonenberg, N. (2006). mTOR signaling: implications for cancer and anticancer therapy. Br J Cancer *94*, 195-199.

Plotnikov, A.N., Schlessinger, J., Hubbard, S.R., and Mohammadi, M. (1999). Structural basis for FGF receptor dimerization and activation. Cell *98*, 641-650.

Presta, M., Dell'Era, P., Mitola, S., Moroni, E., Ronca, R., and Rusnati, M. (2005). Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev *16*, 159-178.

Rizell, M., Andersson, M., Cahlin, C., Hafstrom, L., Olausson, M., and Lindner, P. (2008). Effects of the mTOR inhibitor sirolimus in patients with hepatocellular and cholangiocellular cancer. Int J Clin Oncol *13*, 66-70.

Roskoski, R., Jr. (2007). Vascular endothelial growth factor (VEGF) signaling in tumor progression. Crit Rev Oncol Hematol *62*, 179-213.

Sahin, F., Kannangai, R., Adegbola, O., Wang, J., Su, G., and Torbenson, M. (2004). mTOR and P70 S6 kinase expression in primary liver neoplasms. Clin Cancer Res *10*, 8421-8425.

Scharf, J.G., Dombrowski, F., and Ramadori, G. (2001). The IGF axis and hepatocarcinogenesis. Mol Pathol *54*, 138-144.

Schmitz, K.J., Wohlschlaeger, J., Lang, H., Sotiropoulos, G.C., Malago, M., Steveling, K., Reis, H., Cicinnati, V.R., Schmid, K.W., and Baba, H.A. (2008). Activation of the ERK and AKT signalling pathway predicts poor prognosis in hepatocellular carcinoma and ERK activation in cancer tissue is associated with hepatitis C virus infection. J Hepatol *48*, 83-90.

Schulze, W.X., Deng, L., and Mann, M. (2005). Phosphotyrosine interactome of the ErbB-receptor kinase family. Mol Syst Biol *1*, 2005 0008.

Semela, D., Piguet, A.C., Kolev, M., Schmitter, K., Hlushchuk, R., Djonov, V., Stoupis, C., and Dufour, J.F. (2007). Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. J Hepatol *46*, 840-848.

Shen, Y.C., Hsu, C., and Cheng, A.L. (2010). Molecular targeted therapy for advanced hepatocellular carcinoma: current status and future perspectives. J Gastroenterol *45*, 794-807.

Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., Pestell, R., and Ben-Ze'ev, A. (1999). The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci U S A *96*, 5522-5527.

Siegel, A.B., Cohen, E.I., Ocean, A., Lehrer, D., Goldenberg, A., Knox, J.J., Chen, H., Clark-Garvey, S., Weinberg, A., Mandeli, J., *et al.* (2008). Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol *26*, 2992-2998.

Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., *et al.* (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med *344*, 783-792.

Takahashi, Y., and Nishioka, K. (2011). Therapeutic approaches targeting tumor vasculature in gastrointestinal cancers. Front Biosci (Elite Ed) *3*, 541-548.

Takigawa, Y., and Brown, A.M. (2008). Wnt signaling in liver cancer. Curr Drug Targets *9*, 1013-1024.

Thomas, M.B., and Abbruzzese, J.L. (2005). Opportunities for targeted therapies in hepatocellular carcinoma. J Clin Oncol *23*, 8093-8108.

Thomas, M.B., Chadha, R., Glover, K., Wang, X., Morris, J., Brown, T., Rashid, A., Dancey, J., and Abbruzzese, J.L. (2007). Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer *110*, 1059-1067.

Thompson, M.D., and Monga, S.P. (2007). WNT/beta-catenin signaling in liver health and disease. Hepatology *45*, 1298-1305.

Tovar, V., Alsinet, C., Villanueva, A., Hoshida, Y., Chiang, D.Y., Sole, M., Thung, S., Moyano, S., Toffanin, S., Minguez, B., *et al.* (2010). IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol *52*, 550-559.

Tsai, J.F., Chuang, L.Y., Jeng, J.E., Yang, M.L., Chang, W.Y., Hsieh, M.Y., Lin, Z.Y., and Tsai, J.H. (1997). Clinical relevance of transforming growth factor-beta 1 in the urine of patients with hepatocellular carcinoma. Medicine (Baltimore) *76*, 213-226.

Turner, N., and Grose, R. (2010). Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer *10*, 116-129.

Ueki, T., Fujimoto, J., Suzuki, T., Yamamoto, H., and Okamoto, E. (1997). Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. Hepatology *25*, 619-623.

van der Meel, R., Symons, M.H., Kudernatsch, R., Kok, R.J., Schiffelers, R.M., Storm, G., Gallagher, W.M., and Byrne, A.T. (2011). The VEGF/Rho GTPase signalling pathway: a promising target for anti-angiogenic/anti-invasion therapy. Drug Discov Today *16*, 219-228.

Vanhaesebroeck, B., and Waterfield, M.D. (1999). Signaling by distinct classes of phosphoinositide 3-kinases. Exp Cell Res *253*, 239-254.

Villanueva, A., Chiang, D.Y., Newell, P., Peix, J., Thung, S., Alsinet, C., Tovar, V., Roayaie, S., Minguez, B., Sole, M., *et al.* (2008). Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology *135*, 1972-1983, 1983 e1971-1911.

Wagner, E.F., and Nebreda, A.R. (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer *9*, 537-549.

Wang, W., Chen, J.X., Liao, R., Deng, Q., Zhou, J.J., Huang, S., and Sun, P. (2002). Sequential activation of the MEK-extracellular signal-regulated kinase and MKK3/6-p38 mitogen-activated protein kinase pathways mediates oncogenic ras-induced premature senescence. Mol Cell Biol *22*, 3389-3403.

Wang, Y., Macke, J.P., Abella, B.S., Andreasson, K., Worley, P., Gilbert, D.J., Copeland, N.G., Jenkins, N.A., and Nathans, J. (1996). A large family of putative transmembrane receptors homologous to the product of the Drosophila tissue polarity gene frizzled. J Biol Chem *271*, 4468-4476.

Wehrli, M., Dougan, S.T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature *407*, 527-530.

Whittaker, S., Marais, R., and Zhu, A.X. (2010). The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene *29*, 4989-5005.

Wong, C.M., Fan, S.T., and Ng, I.O. (2001). beta-Catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. Cancer *92*, 136-145.

Wu, A.L., Coulter, S., Liddle, C., Wong, A., Eastham-Anderson, J., French, D.M., Peterson, A.S., and Sonoda, J. (2011). FGF19 regulates cell proliferation, glucose and bile acid metabolism via FGFR4-dependent and independent pathways. PLoS One *6*, e17868.

Wu, X., Ge, H., Gupte, J., Weiszmann, J., Shimamoto, G., Stevens, J., Hawkins, N., Lemon, B., Shen, W., Xu, J., *et al.* (2007). Co-receptor requirements for fibroblast growth factor-19 signaling. J Biol Chem *282*, 29069-29072.

Wu, X., Ge, H., Lemon, B., Vonderfecht, S., Baribault, H., Weiszmann, J., Gupte, J., Gardner, J., Lindberg, R., Wang, Z., *et al.* (2010a). Separating mitogenic and metabolic activities of fibroblast growth factor 19 (FGF19). Proc Natl Acad Sci U S A *107*, 14158-14163.

Wu, X., Ge, H., Lemon, B., Vonderfecht, S., Weiszmann, J., Hecht, R., Gupte, J., Hager, T., Wang, Z., Lindberg, R., *et al.* (2010b). FGF19-induced hepatocyte proliferation is mediated through FGFR4 activation. J Biol Chem *285*, 5165-5170.

Wu, X., and Li, Y. (2009). Role of FGF19 induced FGFR4 activation in the regulation of glucose homeostasis. Aging *1*, 1023.

Wullschleger, S., Loewith, R., and Hall, M.N. (2006). TOR signaling in growth and metabolism. Cell *124*, 471-484.

Xian, W., Schwertfeger, K.L., Vargo-Gogola, T., and Rosen, J.M. (2005). Pleiotropic effects of FGFR1 on cell proliferation, survival, and migration in a 3D mammary epithelial cell model. J Cell Biol *171*, 663-673.

Yamaguchi, R., Yano, H., Iemura, A., Ogasawara, S., Haramaki, M., and Kojiro, M. (1998). Expression of vascular endothelial growth factor in human hepatocellular carcinoma. Hepatology *28*, 68-77.

Yang, Y., Zhou, Y., Lu, M., An, Y., Li, R., Chen, Y., Lu, D.R., Jin, L., Zhou, W.P., Qian, J., *et al.* (2011). Association between fibroblast growth factor receptor 4 polymorphisms and risk of hepatocellular carcinoma. Molecular carcinogenesis.

Yasmin Anum, M.Y., Looi, M.L., Nor Aini, A.H., Merican, I., Wahidah, A., Mohd Radzi, A.H., Nor Azizah, A., and Othman, N.H. (2009). Combined assessment of TGF-beta-1 and alpha-fetoprotein values improves specificity in the diagnosis of hepatocellular carcinoma and other chronic liver diseases in Malaysia. Med J Malaysia *64*, 223-227.

Yoshida, T., Hisamoto, T., Akiba, J., Koga, H., Nakamura, K., Tokunaga, Y., Hanada, S., Kumemura, H., Maeyama, M., Harada, M., *et al.* (2006). Spreds, inhibitors of the Ras/ERK signal transduction, are dysregulated in human hepatocellular carcinoma and linked to the malignant phenotype of tumors. Oncogene *25*, 6056-6066.

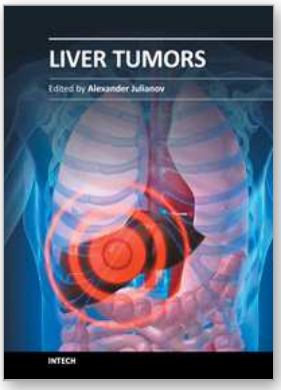
Zarich, N., Oliva, J.L., Martinez, N., Jorge, R., Ballester, A., Gutierrez-Eisman, S., Garcia-Vargas, S., and Rojas, J.M. (2006). Grb2 is a negative modulator of the intrinsic Ras-GEF activity of hSos1. Mol Biol Cell *17*, 3591-3597.

Zeng, G., Awan, F., Otruba, W., Muller, P., Apte, U., Tan, X., Gandhi, C., Demetris, A.J., and Monga, S.P. (2007). Wnt'er in liver: expression of Wnt and frizzled genes in mouse. Hepatology *45*, 195-204.

Zhang, H., Berezov, A., Wang, Q., Zhang, G., Drebin, J., Murali, R., and Greene, M.I. (2007). ErbB receptors: from oncogenes to targeted cancer therapies. The Journal of clinical investigation *117*, 2051-2058.

Zhou, J., Tang, Z.Y., Fan, J., Wu, Z.Q., Li, X.M., Liu, Y.K., Liu, F., Sun, H.C., and Ye, S.L. (2000). Expression of platelet-derived endothelial cell growth factor and vascular endothelial growth factor in hepatocellular carcinoma and portal vein tumor thrombus. J Cancer Res Clin Oncol *126*, 57-61.

Zhu, A.X., Stuart, K., Blaszkowsky, L.S., Muzikansky, A., Reitberg, D.P., Clark, J.W., Enzinger, P.C., Bhargava, P., Meyerhardt, J.A., Horgan, K., *et al.* (2007). Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer *110*, 581-589.



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This book is oriented towards clinicians and scientists in the field of the management of patients with liver tumors. As many unresolved problems regarding primary and metastatic liver cancer still await investigation, I hope this book can serve as a tiny step on a long way that we need to run on the battlefield of liver tumors.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821