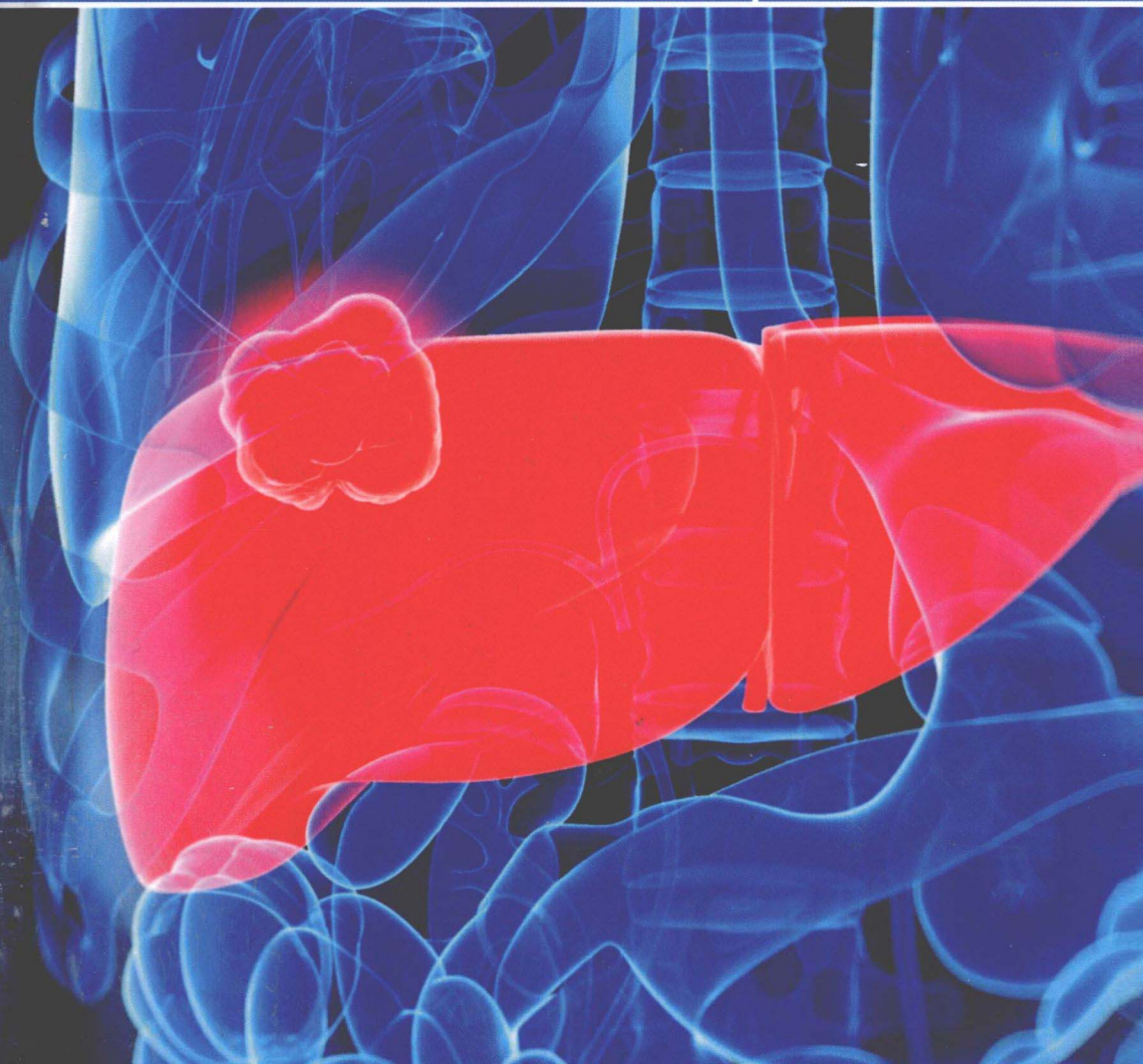


Liver Tumors

Biology, Diagnosis and
Management

Dylan Long



Liver Tumors: Biology, Diagnosis and Management

Edited by **Dylan Long**



New Jersey

Published by Foster Academics,
61 Van Reypen Street,
Jersey City, NJ 07306, USA
www.fosteracademics.com

Liver Tumors: Biology, Diagnosis and Management

Edited by Dylan Long

© 2015 Foster Academics

International Standard Book Number: 978-1-63242-259-0 (Hardback)

This book contains information obtained from authentic and highly regarded sources. Copyright for all individual chapters remain with the respective authors as indicated. A wide variety of references are listed. Permission and sources are indicated; for detailed attributions, please refer to the permissions page. Reasonable efforts have been made to publish reliable data and information, but the authors, editors and publisher cannot assume any responsibility for the validity of all materials or the consequences of their use.

The publisher's policy is to use permanent paper from mills that operate a sustainable forestry policy. Furthermore, the publisher ensures that the text paper and cover boards used have met acceptable environmental accreditation standards.

Trademark Notice: Registered trademark of products or corporate names are used only for explanation and identification without intent to infringe.

Printed in China.

Liver Tumors: Biology, Diagnosis and Management

Preface

The main aim of this book is to educate learners and enhance their research focus by presenting diverse topics covering this vast field. This is an advanced book which compiles significant studies by distinguished experts in the area of analysis. This book addresses successive solutions to the challenges arising in the area of application, along with it; the book provides scope for future developments.

This book is intended for clinicians and scientists in the field of managing patients suffering from liver tumors. As there are many problems related to primary and metastatic liver cancer that are in need of investigation, this book serves as a major step that we are required to travel to be able to fight liver tumors.

It was a great honour to edit this book, though there were challenges, as it involved a lot of communication and networking between me and the editorial team. However, the end result was this all-inclusive book covering diverse themes in the field.

Finally, it is important to acknowledge the efforts of the contributors for their excellent chapters, through which a wide variety of issues have been addressed. I would also like to thank my colleagues for their valuable feedback during the making of this book.

Editor

Contents

	Preface	VII
Chapter 1	Signaling Pathways in Liver Cancer Xinle Wu and Yang Li	1
Chapter 2	Ultrasound Imaging of Liver Tumors – Current Clinical Applications R. Badea and Simona Ioanitorescu	23
Chapter 3	Molecular Genetics and Genomics of Hepatocellular Carcinoma Dilek Colak and Namik Kaya	51
Chapter 4	The <i>Hcs7</i> Mouse Liver Cancer Modifier Maps to a 3.3 Mb Region Carrying the Strong Candidate <i>Ifi202b</i> Andrea Bilger, Elizabeth Poli, Andrew Schneider, Rebecca Baus and Norman Drinkwater	69
Chapter 5	The Role of the Tumor Microenvironment in the Pathogenesis of Cholangiocarcinoma Matthew Quinn, Matthew McMillin, Gabriel Frampton, Syeda Humayra Afroze, Li Huang and Sharon DeMorrow	87
Chapter 6	Liver Tumor Detection in CT Images by Adaptive Contrast Enhancement and the EM/MPM Algorithm Yu Masuda, Tomoko Tateyama, Wei Xiong, Jiayin Zhou, Makoto Wakamiya, Syuzo Kanasaki, Akira Furukawa and Yen Wei Chen	103
Chapter 7	Treatment Strategy for Recurrent Hepatocellular Carcinoma Charing Ching Ning Chong and Paul Bo San Lai	113
Chapter 8	Surgical Strategies for Locally Advanced Hepatocellular Carcinoma Shugo Mizuno and Shuji Isaji	137

Chapter 9	Colorectal Liver Metastasis: Current Management Alejandro Serrablo, Luis Tejedor, Vicente Borrego and Jesus Esarte	145
Chapter 10	Improving the Tumor-Specific Delivery of Doxorubicin in Primary Liver Cancer Junfeng Zhang, Zhen Huang and Lei Dong	175
Chapter 11	Expanding Local Control Rate in Liver Cancer Surgery – The Value of Radiofrequency Ablation Alexander Julianov	189

Permissions

List of Contributors

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

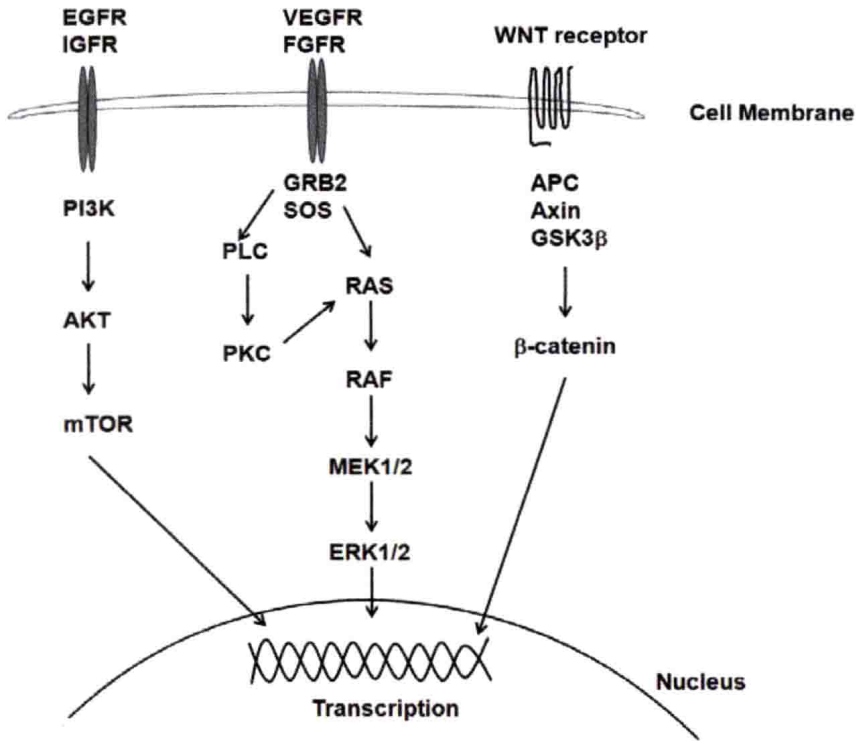


Fig. 1. Major cellular signaling pathways involved in hepatocellular carcinoma tumorigenesis

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 GSK3 β . 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 LRP5 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 GSK3 β that regulate β -catenin degradation (Takigawa and Brown, 2008). Mutations at these positions disrupt recognition by GSK3 β 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 μ m 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-A₁₂₁ and VEGF-A₁₆₅. 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 VEGFR-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 immunoglobulin-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 β Klotho as a co-receptor in activating FGF

receptors. In the presence of β Klotho, 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 tumorigenesis 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 (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 overexpressed 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