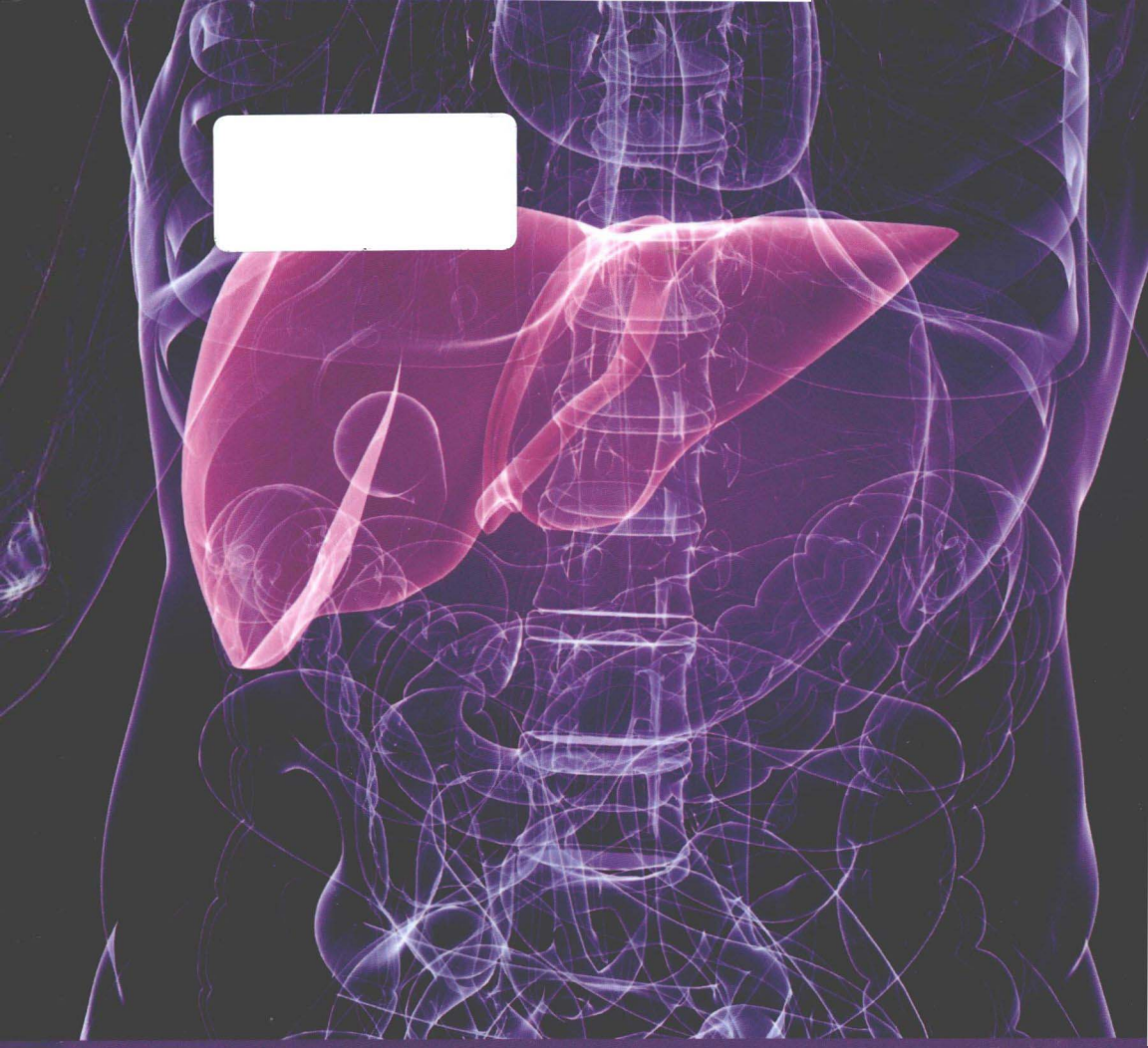

Vital Research in Hepatocellular Carcinoma

Jay Amsel

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Edited by **Jay Amsel**

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Preface

Research-focused information regarding hepatocellular carcinoma has been illustrated in this all-inclusive book. Hepatocellular Carcinoma is a leading cause of cancer death and an important health problem in developing countries where hepatitis B infection is widespread. It has also gained significance with increase in hepatitis C infection in developed countries. Information on hepatocellular carcinoma has developed rapidly. This book is a well detailed publication produced as a result of the efforts of professionals and discusses the most updated information on hepatocellular carcinoma. It essentially deals with the basic research characteristics of hepatocellular carcinoma. Several topics like Biomarkers / Therapeutic Target, Detection / Prevention / Prevalence and Carcinogenesis / Invasion / Metastasis have been elucidated in this book. This book makes vital contributions to the fundamental research of hepatocellular carcinoma. The targeted readers of this book are experts and professionals involved in research on hepatocellular carcinoma. Pathologists, epidemiologists, hospital managers and drug producers will also find this book relevant as a handy source of reference.

This book is a comprehensive compilation of works of different researchers from varied parts of the world. It includes valuable experiences of the researchers with the sole objective of providing the readers (learners) with a proper knowledge of the concerned field. This book will be beneficial in evoking inspiration and enhancing the knowledge of the interested readers.

In the end, I would like to extend my heartiest thanks to the authors who worked with great determination on their chapters. I also appreciate the publisher's support in the course of the book. I would also like to deeply acknowledge my family who stood by me as a source of inspiration during the project.

Editor

Contents

Part 1

Biomarker / Therapeutic Target

1

Novel Therapeutic Targets for Hepatocellular Carcinoma Treatment

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Surgery is a possible curative treatment, but most symptomatic HCC cases are in advanced stage where surgical resection is not possible. For this group of patients, the prognosis after any kind of therapy remains unsatisfactory due to high relapse rate (Llovet et al., 2003). Studies were rigorously conducted to tackle various obstacles in treating HCC, putting the focuses on targeting cancer cells that either disseminated from the tumor origin, or escaped from therapeutic effects. Recently, a multikinase inhibitor sorafenib was approved by FDA for the treatment of advanced HCC patients. It marks a major advance in the field as the first efficacious targeted therapy for HCC. The primary molecular targets of sorafenib include vascular endothelial factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR) and Raf (Wilhelm et al., 2004). Although it significantly prolongs both patient survival and the time to progression, its overall survival benefit is modest (Llovet et al., 2008).

Other HCC associated targets, such as epidermal growth factor (EGF) signaling (Hampton, 2007), telomerase (Djojosubroto et al., 2005) and cyclooxygenase (Márquez-Rosado et al, 2005), were studied intensively with regard to their therapeutic effects. However, the benefits are far from satisfactory, so there is still a need to identify new therapeutic targets. The exploration of new targets against HCC involves multiple disciplines including hepatology, oncology, pathology and molecular studies. Increasing number of therapeutic targets which play crucial roles in HCC were identified. Identification of new targets not only improves the current HCC therapeutic modality, but also drives a deeper understanding of HCC that allows personalized treatment in the future. In this chapter, we will briefly review the novel molecular and cellular players that contribute to HCC tumorigenesis and progression, and evaluate their potential as additional therapeutic targets.

2. Growth receptor signaling

The studies of sorafenib administration and other growth signaling inhibitors demonstrated the prowess of targeting growth signalings such as epidermal growth factor (EGF), VEGF and PDGF pathways. In HCC, many other growth signalings were identified that markedly contributes to tumorigenesis and pathogenesis. They include insulin-like growth factor

signaling and mTOR pathway, and numerous studies suggested these pathways can be the targets against HCC.

2.1 Insulin-like growth factor signaling

The insulin-like growth factor (IGF) signaling pathway is frequently dysregulated in HCC. The activation of IGF signaling can be established in malignant cells through an autocrinal route when the activated signaling is induced by an overexpressed IGF ligand in HCC cells (Nussbaum et al., 2008). Insulin-like growth factor 2 (IGF-2) is increased after an inflammatory response to liver damage or viral transactivation (Feitelson et al., 2004), and it is the major ligand contributing to the increased IGF activity in HCC. IGF-2-mediated induction of IGF signaling is prevalent in human HCC, where IGF-2 is overexpressed in 16-40%, whilst the level of competitive receptor for IGF-2 is decreased in around 80% (Whittaker et al., 2010). As such, IGF receptor-ligand binding is enhanced, and subsequent downstream signaling is activated in cancer cells. Activation of IGF signaling in HCC cells is associated with increase of cell proliferation rate (Schirmacher et al, 1992). While RNAi-mediated knockdown of IGF-2 could reduce the cell proliferation and induce apoptosis in HCC cells, small molecule inhibiting IGF-2-dependent IGF signaling was able to impair the growth of HCC cells and retard tumour growth in mice xenograft (Lund et al., 2004).

Altered IGF-2 bioavailability is another reason for the hyperactivation of IGF signaling in HCC. Normally, circulating IGF-2 is bound by IGF-binding protein (IGFBP) so that the efficiency of ligand-receptor binding is lowered. In HCC, members of IGFBPs are downregulated so that less IGF-2 is sequestrated which allow uncontrolled IGF-2-receptor interaction (Hanafusa et al, 2002). Hence, reducing the level of IGF-2 in circulation is another valid approach to abrogate the IGF signaling. Re-introduction of recombinant human IGFBP-3 was tested and showed potent effect in lowering the activity of IGF-2 (Aishima et al., 2006). IGFBP-3 was able to inhibit cancer cell growth and attenuate mitogenic activity of HCC cells. It is also reported that IGFBP-3 decreased the phosphorylation and activity of numerous pro-tumorigenic proteins such as IRS-1, MAPK, Elk-1, Akt-1 and phosphatidylinositol 3'-kinase (Huynh et al., 2002).

In addition, inhibition of IGF signaling can also be achieved by disrupting other players along the IGF signaling axis. IGF signal transduction is mediated by the Insulin receptor, IGF-IR and a hybrid of both receptors. In HCC, there is detectable level of IGF receptors ready for the signal generation stimulated by the overexpressed IGF-2. Studies showed that blocking of the receptors was able to give antitumoral effect in HCC cells (Nussbaum et al., 2008). Selective blockage of IGF-IR by monoclonal antibody effectively disrupted IGF signaling, reduced cell viability and proliferation. The inhibition of IGF-IR signal initiation was able to delay tumor growth and prolonged survival in vivo (Tovar et al., 2010). With understanding of IGF signaling mechanism in HCC, it is possible to employ various strategies to effectively inhibit IGF signaling, and in turn suppress cell proliferation and increase apoptosis in HCC.

2.2 mTOR pathway

mTOR pathway is a downstream growth signal induced by EGF and IGF signaling, and is coupled with PI3K/AKT pathway. mTOR pathway has an important role in the

pathogenesis of HCC, where aberration of mTOR pathway was seen in 15% to 41% of HCC cases ranged from 15% to 41% (Hu et al., 2003). In HCC, the commonly hyperactive EGF and IGF signaling is responsible for the induction of PI3K/AKT/mTOR pathway, promoting tumor progression. The mTOR signaling is mediated by mTOR complex 1 and 2 (mTORC1 and mTORC2). mTORC1 is comprised of mTOR, regulatory associated protein of mTOR (RAPTOR), and mammalian LST8/G-protein β-subunit-like protein. mTORC1 is a downstream signal of AKT, and has a pivotal role in regulating cell growth and proliferation. mTORC1 activates S6 kinase to regulate protein synthesis and induces cell cycle to proceed from G1 to S phase (Bjornsti and Houghton, 2004).

Besides, mTOR is also the subunit of mTORC2 which consists of a protein RAPTOR-independent companion of mTOR (RICTOR), and proline-rich protein 5/G-protein β-subunit-like protein. Unlike mTORC1 which is inducible by AKT, mTORC2 plays a critical role in the phosphorylation and activation of AKT (Sarbassov et al., 2005). The serine/threonine kinase AKT acts as a cytoplasmic regulator of numerous signals. It is shown that AKT is frequently amplified and overexpressed in various cancers, and it demonstrates significant oncogenic properties in diverse cancer types. In homeostasis condition, AKT is negatively regulated by the tumor-suppressor PTEN. However, increased activation of AKT is often observed, because PTEN is frequently lost in cancers including HCC. Other than mTORC1, AKT regulates a wide-spectrum of targets such as cyclin D1 and MDM2/p53 (Vivanco & Sawyers, 2002). In HCC, aberration of mTORC2 enhances AKT activity, induces downstream AKT targets and promotes tumorigenesis. One can see that the AKT regulating effect of mTORC2 is as important as mTORC1 within the PI3K/AKT/mTOR pathway.

Recently, it is suggested that the PI3K/AKT/mTOR pathway can be a major molecular target in cancer remedy. As a critical player in the mTOR signaling, the activity of mTOR often increases in HCC. Blockage of mTOR-mediated signaling showed antineoplastic activity in different experimental models of HCC. The use of mTOR inhibitors could reduce cell proliferation in vitro, and decrease tumor growth in xenografted mouse model (Villanueva et al., 2008). mTOR inhibitors such as sirolimus and everolimus demonstrated potent antitumor properties. Encouraging results were obtained when both mTOR inhibitors were studied in clinical trials, either as a single agent or as adjuvant. Furthermore, components in the mTOR complexes can also be the therapeutic targets. High level of RICTOR is correlated to early recurrence in HCC, and siRNA knockdown of RICTOR reduces HCC cells viability (Villanueva et al., 2008). Disruption of mTOR complexes might have additive benefit along with mTOR inhibition to abrogate mTOR pathway in treating HCC.

3. Cell-surface protein

3.1 Glypican-3

Glypican-3 (GPC3) is a protein anchored to the cell surface by a glycosyl-phosphatidylinositol link. Glypican-3 is highly expressed in HCC, and plays a role in stimulating various tumorigenic signaling pathways. GPC3 is specifically expressed in HCC, but not in cholangiocarcinoma or normal liver tissue. More than 70% of HCC tumors were observed with high GPC3 level compared to normal liver tissues (Hsu et al., 1997). Consistent with the high GPC3 protein expression found in clinical samples, numerous

HCC cell lines have high expression level of GPC3 (Midorikawa et al., 2003). In addition, GPC3 expression is correlated with the prognosis of HCC, where GPC3-positive HCC patients have a significantly lower 5-year survival rate than patients who are GPC3-negative (Shirakawa et al., 2009).

One of the GPC3 tumorigenic roles is the activation of Wnt/β-catenin signaling. It is shown that GPC3 is able to interact with Wnt ligands, and induces canonical Wnt-signaling to trigger the stabilization of β-catenin and induction of cyclin D1 (Capurro et al., 2005). The heparin sulfate chain of GPC3 is reported to bind with basic growth factors such as FGF-2. The interaction between GPC3 and FGF-2 is frequently observed in HCC cells, and is responsible for phosphorylation of ERK and AKT (Midorikawa et al., 2003). This interaction plays a role in the increase of HCC cell proliferation, and growth of tumor in nude mouse model. Additionally, GPC3 interplays with hedgehog signaling in regulating developmental growth (Capurro et al., 2008). Though yet to be elucidated, the GPC3-hedgehog signaling is suggested to contribute to HCC development.

Targeting GPC3 and its related growth signaling is a relevant approach to inhibit HCC growth. Inhibition of the interaction between GPC3 and Wnt or FGF-2 should theoretically reduce HCC growth (Capurro et al., 2005; Midorikawa et al., 2003). GPC3 is also a useful target in immunotherapy against HCC. The therapeutic monoclonal antibody against GPC3 has been developed which could induce antibody-dependent HCC cytotoxicity. Targeting GPC3 is able to inhibit tumor growth of HCC cell line xenograft (Ishiguro et al., 2008). Study also showed the concomitant treatment with GPC3 monoclonal antibody and sorafenib was more potent in preventing tumor growth than sorafenib alone in the HepG2 xenograft model (Ishiguro et al., 2008). It is likely that targeting GPC3 could provide great clinical benefit during HCC management.

3.2 Cadherin 17

Cadherins are important cell adhesion molecules strongly associated with cancer progression. Downregulation of E-cadherin (Du et al., 2009) and overexpression of P-cadherin are often observed in advanced tumor which processes crucial cellular event like epithelial-mesenchymal transition (Sun et al., 2011). Cadherin 17 (CDH17) is another adhesion molecule upregulated in HCC, and it is linked to the tumorigenesis in various gastrointestinal regions (Wang et al., 2005). The upregulation of CDH17 is capable of transforming premalignant liver progenitor cells into liver carcinomas in mice. While forced expression of CDH17 promoted tumor growth from hepatic progenitor cells, silencing of CDH17 reduced the aggressiveness of metastatic HCC cells (Liu et al., 2009). Knockdown of CDH17 by RNA-interference decreased the proliferation rate of HCC cell lines despite their metastatic potential in vitro and in vivo. It is shown that targeting CDH17 can concurrently inactivate Wnt/β-catenin signaling and reduce cyclin D1 level, leading to both growth inhibition and cell death. Inhibition of CDH17 results in the re-localization of nuclear β-catenin to the cytoplasm so as to attenuate the Wnt/β-catentin signaling (Liu et al., 2009).

Multiple isoforms of CDH17 protein are present in the HCC samples, and it is found that the isoform lacking exon 7 is the most abundant in HCC samples (Wang et al., 2005). CDH17 isoform lacking exon 7 cannot be found in normal liver tissue whereas it is present in about 50% of human HCC and 30% of premalignant tissues. Detection of this CDH17 isoform was