Molecular Pathology Library
Series Editor: Philip T. Cagle

Chen Liu
Editor

Precision Molecular Pathology of Liver Cancer

Springer

Molecular Pathology Library

Series Editor:

Philip T. Cagle
Houston, TX
USA

More information about this series at http://www.springer.com/series/7723

Chen Liu
Editor

Precision Molecular Pathology of Liver Cancer

Springer

Editor
Chen Liu
Department of Pathology and Laboratory Medicine
Rutgers Robert Wood Johnson Medical School
and New Jersey Medical School
Newark, New Jersey
USA

ISSN 1935-987X ISSN 1935-9888 (electronic)
Molecular Pathology Library
ISBN 978-3-319-68080-4 ISBN 978-3-319-68082-8 (eBook)
https://doi.org/10.1007/978-3-319-68082-8

Library of Congress Control Number: 2017962859

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Hepatocellular carcinoma (HCC) is the predominant primary malignant cancer in the liver. It is one of the most common and malignant cancers in the world. There are 700,000 deaths due to HCC every year. The cancer incidence is increasing in many countries, including the United States. Unfortunately, the treatment options are very limited compared to other human cancers. Many clinical trials have been conducted over the years, but the results are generally disappointing. The high failure rate for clinical trials is partially attributed to lack of adequate biomarkers for patient selection. Developing molecular markers is paramount for early diagnosis and optimal treatment of HCC. This book provides the most updated knowledge on the advancement of molecular pathogenesis, molecular diagnosis, and therapy development. The authors are experts in the topics they have contributed. Besides reviewing the current available knowledge, the authors also discuss their prospective for future developments in precision/personalized medicine approach for HCC.

Newark, New Jersey, USA — Chen Liu

Contents

Contributors

Naziheh Assarzadegan, M.D. Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Cuong Bach, Ph.D. Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, USA

Tony S. Brar, M.D. Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, FL, USA

Roniel Cabrera, M.D. Division of Gastroenterology, Hepatology, and Nutrition, University of Florida, Gainesville, FL, USA

Darren R. Carpizo, M.D. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Joydeep Chakraborty, M.D. Division of Gastroenterology, Hepatology, and Nutrition, University of Florida, Gainesville, FL, USA

Amy Leigh Collinsworth, M.D. Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Dan Delitto, M.D. Department of Pathology and Laboratory Medicine, Rutgers New Jersey Medical School and Robert Wood Johnson Medical School, Newark, NJ, USA

Michael Feely, M.D. Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL, USA

Joseph R. Grajo, M.D. Department of Radiology, University of Florida, Gainesville, FL, USA

Stephanie H. Greco, M.D. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Eric Hilgenfeldt, M.D. Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, FL, USA

Division of Gastroenterology, Department of Internal Medicine, Carolinas Medical Center, Charlotte, NC, USA

Jeannette Huaman, Ph.D. Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, USA

Department of Biology, The Graduate Center of The City University of New York, New York, NY, USA

Adeodat Ilboudo, Ph.D. Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, USA

Junfang Ji, Ph.D. Life Sciences Institute, Zhejiang University, Hangzhou, China

Jesse Kresak, M.D. Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Niya Liu, Ph.D. Life Sciences Institute, Zhejiang University, Hangzhou, China

Chen Liu, M.D., Ph.D. Department of Pathology and Laboratory Medicine, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ, USA

Department of Pathology and Laboratory Medicine, Rutgers New Jersey Medical School and Robert Wood Johnson Medical School, Newark, NJ, USA

Olorunseun O. Ogunwobi, M.D., Ph.D. Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, USA

Department of Biology, The Graduate Center of The City University of New York, New York, NY, USA

Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, Cornell University, New York, NY, USA

Kien Pham, Ph.D. Department of Pathology and Laboratory Medicine, Rutgers New Jersey Medical School and Robert Wood Johnson Medical School, Newark, NJ, USA

Williams Puszyk, Ph.D. Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Keith Robertson, Ph.D. Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Rochester, MN, USA

Naziya Samreen, M.D. Department of Radiology, University of Florida, Gainesville, FL, USA

Consuelo Soldevila-Pico, M.D. Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, FL, USA

Kristen Spencer, M.D. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Beau Toskich, M.D. Department of Radiology, University of Florida College of Medicine, Gainesville, FL, USA

Xin Wei Wang, M.D., Ph.D. Liver Carcinogenesis Section, Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Xiyang Wei, Ph.D. Life Sciences Institute, Zhejiang University, Hangzhou, China

Lanjing Zhang, M.D., M.S., F.C.A.P., F.A.C.G. Department of Pathology, University Medical Center of Princeton, Plainsboro, NJ, USA

Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA

Faculty of Arts and Sciences, Department of Biological Sciences, Rutgers University, Newark, NJ, USA

Etiology and Pathogenesis of Hepatocellular Carcinoma

1

Tony S. Brar, Eric Hilgenfeldt, and Consuelo Soldevila-Pico

1.1 Introduction

Worldwide, hepatocellular carcinoma (HCC) ranks as the third leading cause of cancer-related deaths [1, 2]. Historically, HCC has been more prevalent in the developing world; however, in the last two decades the incidence has nearly doubled in developed countries; this has been largely due to liver cirrhosis [2, 3]. The 5-year survival rate of HCC in the United States is only 8.9% [4]. Even with aggressive conventional therapy, this malignancy is the second most lethal cancer after pancreatic adenocarcinoma [4]. This review summarizes the etiology and pathogenesis of HCC.

1.2 Etiology

HCC has been associated with various risk factors including viral hepatitis, cirrhosis (with any underlying etiology including nonalcoholic fatty liver disease (NAFLD)), and toxin-mediated disease (Fig. 1.1). There are two main hepatitis viruses associated with the development of HCC: hepatitis B virus (HBV) and hepatitis C virus (HCV) [5]. The major toxins that predispose to HCC include alcohol and aflatoxin-B1 [6]. During the last 10 years, there has been a clear delineation of the nature of the genetic alterations in HCC, including homozygous deletions in chromosome 9 (CDKN2A) and high-level DNA amplifications in chromosome 11q13 (FGF19/CNND1) and 6p21 (VEGFA) [7]. Associated with an increased telomerase expression, the most frequent mutations affect TERT promoter [7]. CTNNB1 and TP53 are the next most prevalent mutations [7]. Other etiological factors have been proposed to develop into HCC but at a much lower frequency.

T.S. Brar, M.D. • E. Hilgenfeldt, M.D. • C. Soldevila-Pico, M.D. (✉)
Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, FL, USA
e-mail: Tony.Brar@medicine.ufl.edu; Consuelo.SoldevilaPico@medicine.ufl.edu

C. Liu (ed.), *Precision Molecular Pathology of Liver Cancer*,
Molecular Pathology Library, https://doi.org/10.1007/978-3-319-68082-8_1

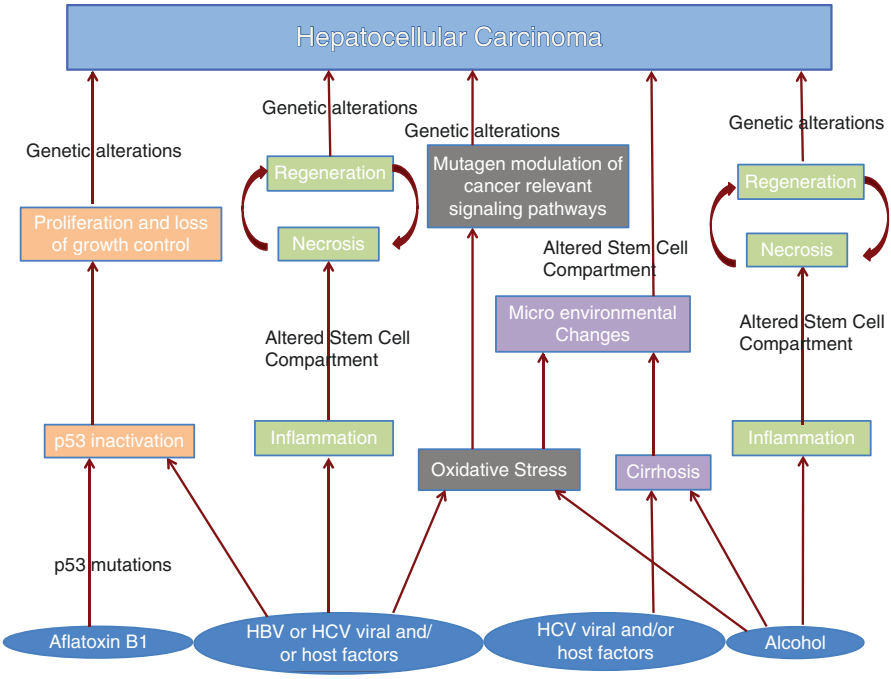

Fig. 1.1 Hepatocarcinogenesis mechanisms. The various risk factors are shown. Hepatitis B virus (HBV) and hepatitis C virus (HCV)

1.3 Virus-Induced Hepatocarcinogenesis

As mentioned previously, the two major viral contributors to HCC are HBV and HCV. Worldwide, HBV infects over two billion individuals and accounts for more than 300,000 deaths annually [8], and close to half of all HBV-related deaths are attributable to HCC [8]. A strong correlation between elevated serum HBV DNA levels and incidence of HCC has been described [9]. The prevalence of HCV is much lower than that of HBV [10]. There are over 170 million individuals worldwide infected with HCV [11], but only 2.5% develop HCC. However, over 20% of chronic cases result in liver cirrhosis [12]. Both viral and host factors are involved in driving hepatocarcinogenesis.

1.3.1 HBV

Classified as a member of the *Hepadnaviridae* family, HBV is a partially double-stranded hepatotropic DNA virus [13]. During the transformation process, there is direct involvement of HBV. To illustrate, HBV genomic integration has been linked to host DNA microdeletions, which target cancer-related genes such as

mitogen-activated protein kinase 1 (MAPK1), platelet-derived growth factor receptor-beta (PDGFR-β), and telomerase reverse transcriptase (TERT) [14]. There are several other mechanisms that have demonstrated the direct involvement of HBV in the development of HCC [15]. The expression of growth control genes (JNK, ERK, Raf, Ras, MAPK, and SRC tyrosine kinases) can be altered by protein x (HBx) transcriptional activation [16]. Lastly, tumor-suppressor p53 can be bound and inactivated in vitro by HBx; this compromises DNA damage checkpoints and increases cellular survival and proliferation [17].

There are several ways in which host-viral interactions play a role. HBV mutations may result in retention of the virus within the hepatocyte, allowing the virus to escape the host's system, leading to liver disease [18]. An alternative mechanism involves the generation of free radicals which activate stellate cells through the induction of oxidative stress, thus stimulating survival-signaling pathways [19] creating a pro-carcinogenic state in the liver. Most HBV infections are acute; however, 10% of adults have reduced clearance leading to chronic active infection [20]. This creates sustained cycles of necrosis-inflammation-regeneration [4]. This process can lead to genomic instability through the propagation of oncogenic lesions and telomere erosion [21].

1.3.2 HCV

As a member of the *Flaviviridae* family, HCV is a non-cytopathic positive-stranded RNA genome [22]. There are several important distinctions between HCV and HBV that are relevant to hepatocarcinogenesis. First, HCV is a RNA virus so it cannot integrate into host genomes as it has no DNA intermediate [23]. Second, HCV is much more likely to yield chronic infection: 80% of HCV vs. 10% of HBV [24]. This can be attributed to high rates of replication errors, which result in immune avoidance by HCV [25]. Lastly, after 10 years of infection, about 10% of HCV-infected patients develop liver cirrhosis, a percentage that is almost 20 times larger than that of HBV-infected patients [24].

Core proteins and HCV RNA impair important steps involved with T-cell activation and dendritic cell functions [26]. NS5A nonstructural protein and HCV core protein are involved with evasion from immune-mediated cell killing [27]. This process involves interactions with various factors that include but are not limited to tumor necrosis factor-alpha (TNF-α) receptor and interferon-alpha (IFNα) [28]. Furthermore, NS3 and NS4A HCV proteins cleave and activate components through their protease function that is vital in signaling an immune response [29, 30]. HCV core proteins have been shown to modulate cell proliferation by interacting with components of the MAPK signaling pathway which includes Raf, MEK, and ERK [31]. The p53-regulated pathways that control tumor angiogenesis, cell-cycle progression, response to hypoxic and genotypic stresses, and cellular survival are inactivated by NS5A through sequestration of the perinuclear membrane [32, 33]. An oxidative stress-mediated mechanism is likely involved with HCV-induced HCC due to the carcinogenic potential of the HCV core proteins that lead to hepatic steatosis [34].

1.4 NAFLD Cirrhosis-Induced Hepatocarcinogenesis

The rise in NAFLD can be associated with the increase in the prevalence of diabetes mellitus and obesity [6]. It has been estimated that close to two thirds of the diabetic and obese population ultimately develop NAFLD [35]. Globally, the most common etiology for chronic liver disease is NAFLD [35]. NAFLD can be viewed as a spectrum of disease ranging from an accumulation of fat greater than 5% of liver weight known as simple steatosis to an aggressive form with fibrosis and necroinflammation nonalcoholic steatohepatitis (NASH) [36]. Up to 20% of the patients who develop NASH are likely to advance to cirrhosis and are at risk for complications of end-stage liver disease [37]. One of these complications is HCC.

There are numerous mechanisms underlying the pathogenesis of NASH-related HCC (Fig. 1.2). Pro-inflammatory cytokines including IL-6 and TNF-α and free fatty acids are produced with insulin resistance which is associated with NAFLD [38]. Pro-oncogenic pathways are promoted by TNF-α that specifically involve mammalian target of rapamycin complex (mTOR), c-Jun amino acid-terminal kinase (JNK), and nuclear factor κB [39, 40]. A decreased carcinogenic response occurs with weight loss through reduced levels of IL-6 and TNF-α [41]. Continued malignant transformation is likely with prolonged upregulation of the IL-6/STAT3 axis [42].

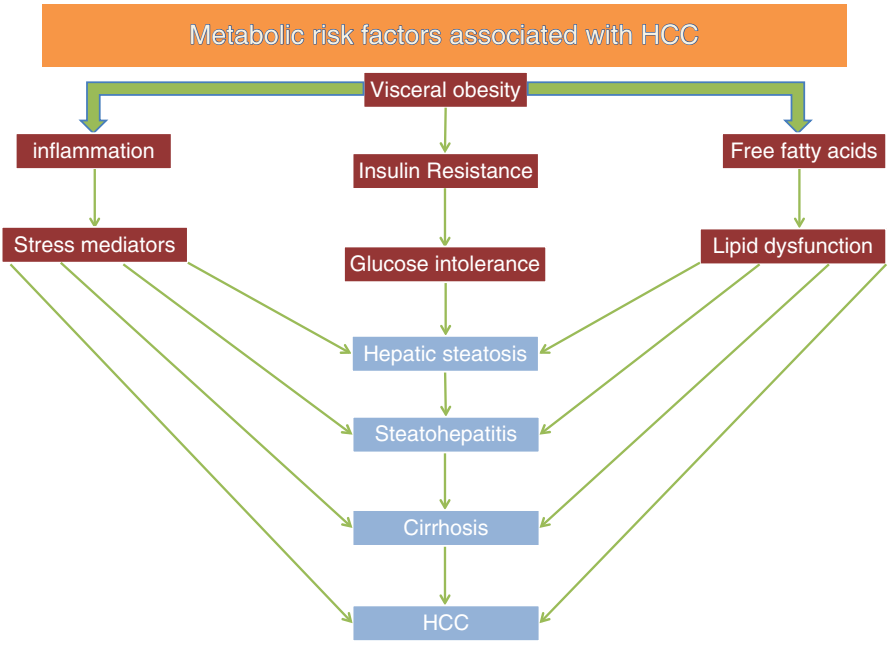

Fig. 1.2 Metabolic pathogenetic pathways to hepatocellular carcinoma (HCC)

Insulin-like growth factor-1 (IGF-1) is produced through the upregulation by insulin resistance [43]. HCC development is linked to IGF-1-promoted processes such as activating mitogen-activated protein kinases (MAPK) and the expression of proto-oncogenes c-jun and c-fos in vitro [43]. A MAPK, JNK, is downregulated by weight loss [44]. The role of phosphorylated JNK in the development of HCC is demonstrated by histopathological analysis revealing that over 70% of HCC tissue specimens stain positive for the protein kinase [44]. The frequency of TERT promoter mutations rapidly increased during the different steps of the transformation of premalignant lesions into HCC on cirrhosis [45]. Consequently, somatic TERT promoter mutation is a new biomarker predictive of transformation of premalignant lesions into HCC [45].

1.5 Toxin-Mediated Hepatocarcinogenesis

1.5.1 Alcohol Induced

Chronic alcohol use is an important risk factor for the development of HCC [46]. Chronic alcohol use causes activation of monocytes through the production of inflammatory cytokines [47]. Circulating endotoxin concentrations are increased, activating Kupffer cells and resulting in the release of many cytokines and chemokines (including prostaglandin E2, IL6, TNF-α, interleukin-1β); these factors have an adverse effect on the survival of hepatocytes [48]. An increased sensitivity to TNF-α in the setting of chronic alcohol exposure leads to stellate cell activation, chronic hepatocyte destruction-regeneration, cirrhosis, and eventually HCC [49].

Other alcohol-induced oxidative stress mechanisms include changes in hepatocarcinogenesis signaling pathways with the loss of protective effects of IFNγ, reduced STAT1 (signal transducer and activator of transcription 1) tyrosine phosphorylation, and diminished STAT1-directed activation of IFNγ signaling, which result in subsequent hepatocyte damage [50]. Fibrosis and/or cirrhosis can be the result of oxidative stress [51] creating a permissive HCC microenvironment that has a pro-carcinogenic effect which has been shown in PDGF transgenic mice [52]. The fibrotic response involves elevated collagen synthesis and cell proliferation that occurs with oxidative stress induction of cultured stellate cells with isoprostane treatment [53]. In the injured liver, the main source of collagen deposition are the stellate cells [54].

1.5.2 Aflatoxin-B1 Induced

An increased risk for the development of HCC also occurs with ingestion of the fungal toxin aflatoxin-B1 [55]. Cooperating mutational activation of oncogenes such as HRAS and associated with a particular p53 mutation, aflatoxin-B1 functions as a specific mutagen [56]. The major difference between this etiology and