Molecular and Translational Medicine
Series Editors: William B. Coleman · Gregory J. Tsongalis

Yujin Hoshida *Editor*

Hepatocellular Carcinoma

Translational Precision Medicine Approaches

Humana Press

Molecular and Translational Medicine

Series Editors:
William B. Coleman
Department of Pathology and Lab Medicine
University of North Carolina School of Medicine
Chapel Hill, NC, USA

Gregory J. Tsongalis
Department of Pathology and Lab Medicine
Dartmouth-Hitchcock Medical Center
Lebanon, NH, USA

As we enter into this new era of molecular medicine with an expanding body of knowledge related to the molecular pathogenesis of human disease and an increasing recognition of the practical implications for improved diagnostics and treatment, there is a need for new resources to inform basic scientists and clinical practitioners of the emerging concepts, useful applications, and continuing challenges related to molecular medicine and personalized treatment of complex human diseases. This series of resource/reference books entitled Molecular and Translational Medicine is primarily concerned with the molecular pathogenesis of major human diseases and disease processes, presented in the context of molecular pathology, with implications for translational molecular medicine and personalized patient care.

More information about this series at http://www.springer.com/series/8176

Yujin Hoshida
Editor

Hepatocellular Carcinoma

Translational Precision Medicine Approaches

Humana Press

Editor
Yujin Hoshida
Department of Internal Medicine
University of Texas Southwestern Medical Center
Dallas, TX
USA

ISSN 2197-7852 ISSN 2197-7860 (electronic)
Molecular and Translational Medicine
ISBN 978-3-030-21539-2 ISBN 978-3-030-21540-8 (eBook)
https://doi.org/10.1007/978-3-030-21540-8

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

Preface

The recent enormous advances in biotechnology, therapeutic development strategy, and information technology have led to the emergence of precision medicine approach, aiming to optimize patient care according to characteristics and needs of each individual and subpopulation. This paradigm shift is relevant to hepatocellular carcinoma (HCC) care because of the highly complex and heterogeneous clinical demographics, natural history, and molecular pathogenesis across the patients. Furthermore, HCC prognosis is still dismal, and its incidence keeps rising in multiple regions, such as North America, in parallel with the epidemic of obesity and metabolic syndrome. Thus, there is a mounting expectation for introducing the precision medicine concept in the HCC care to make a significant impact on this growing global health issue.

Studies have been elucidating promising therapeutic targets and developing useful tools that will help introduce new tailored approaches in HCC screening, diagnosis, treatment, and prevention, which have potential to transform clinical care of HCC patients. These achievements represent unprecedented opportunities to clinically implement the novel strategies with the rapidly expanding biomedical and IT infrastructure and resources. The major challenge is to coordinate the efforts across highly multidisciplinary and diverse expertise involved in the HCC care and research, including clinical hepatology, surgical oncology, diagnostic and interventional radiology, population science, high-throughput omics, systems biology, nanomedicine, biomarker, molecular targeted therapies, and experimental modeling.

This book was planned to assist the collaboration between the diverse disciplines and facilitate forward and reverse translation between basic and clinical research by providing a comprehensive overview of relevant areas, covering epidemiological trend and population-level patient management strategies (Chaps. 1, 2, and 3), new diagnostic and prognostic tools (Chaps. 4, 5, 6, and 7), recent advances in the standard care and novel therapeutic approaches (Chaps. 8, 9, 10, 11, 12, and 13),

and new concepts in pathogenesis and experimental approaches and tools (Chaps. 14, 15, and 16), by the leaders in the respective fields. The editor hopes that this book helps promote the development of personalized precision care strategies that leads to substantial improvement of disease burden and patient prognosis in HCC.

Dallas, TX, USA
February 2019

Yujin Hoshida

Acknowledgments

The editor would like to thank the authors for contributing the up-to-date and thoughtfully written chapters. The editor would also like to thank the following funding supports: Cancer Prevention and Research Institute of Texas (RR180016), US National Institute of Diabetes and Digestive and Kidney Diseases (R01DK099558), European Commission (ERC-2014-AdG-671231), and Irma T. Hirschl Trust.

Contents

Part III Treatment

Part IV Molecular Pathogenesis and Therapeutic Discovery

Contributors

Sai Krishna Athuluri-Divakar Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Thomas F. Baumert Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Pôle Hépato-digestif, Institut Hopitalo-Universitaire, Hôpitaux Universitaires, Strasbourg, France

Irun Bhan Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA

Alyson P. Black Department of Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA

Zachary J. Brown Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Qiushi Chen Harold and Inge Marcus Department of Industrial and Manufacturing Engineering, Pennsylvania State University, University Park, PA, USA

Jagpreet Chhatwal Institute for Technology Assessment, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Raymond T. Chung Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Ian R. Corbin Advanced Imaging Research Center, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Department of Internal Medicine, Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Derek J. Erstad Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Bryan C. Fuchs Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Naoto Fujiwara Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Tim F. Greten Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Daniel A. Haber Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA

Stefanie Hectors Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Caitlin A. Hester Division of Surgical Oncology, Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX, USA

Yujin Hoshida Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Man Hsin Hung Laboratory of Human Carcinogenesis, Bethesda, MD, USA

Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan

School of Medicine, National Yang-Ming University, Taipei, Taiwan

Binu V. John Department of Medicine, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA, USA

Sanjeeva P. Kalva Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Department of Radiology, Massachusetts General Hospital, Boston, MA, USA

Richard D. Kim Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA

Anuhya Kommalapati Department of Internal Medicine, University of South Carolina School of Medicine, Columbia, SC, USA

Daniel Lamus Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Sung Hwan Lee Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Ju-Seog Lee Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Sara Lewis Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Junjie Li Advanced Imaging Research Center, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Shen Li Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Po-Hong Liu Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Amaia Lujambio Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Graduate School of Biomedical Sciences at Icahn School of Medicine at Mount Sinai, New York, NY, USA

Joachim Lupberger Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Amit Mahipal Department of Medical Oncology, Mayo Clinic, Rochester, MN, USA

Anand S. Mehta Department of Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA

Rutika Mehta Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA

Pedro Molina-Sánchez Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Neehar D. Parikh, MD MS Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

Anjana Pillai Department of Internal Medicine, University of Chicago, Chicago, IL, USA

Allen A. Razavi Tufts University School of Medicine, Boston, MA, USA

Nicole E. Rich Division of Digestive and Liver Diseases, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA

Natascha Roehlen Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Daniela Said Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Universidad de los Andes, Santiago, Chile

Antonio Saviano Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Pôle Hépato-digestif, Institut Hopitalo-Universitaire, Hôpitaux Universitaires, Strasbourg, France

Jae-Jun Shim Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Amit G. Singal, MD, MS Division of Digestive and Liver Diseases, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA

Armando Andres Roca Suarez Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Patrick D. Sutphin Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Kenneth K. Tanabe Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Bachir Taouli Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Ryosuke Tateishi Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

David T. Ting Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA

Naik Vietti Violi Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Lausanne University hospital and University of Lausanne, Lausanne, Switzerland

Alessia Virzì Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Xin Wei Wang Laboratory of Human Carcinogenesis, Bethesda, MD, USA

Liver Cancer Program, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA

Connor A. West Department of Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA

Sun Young Yim Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Adam C. Yopp Division of Surgical Oncology, Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX, USA

Shijia Zhu Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Yueran Zhuo Institute for Technology Assessment, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Part I
Clinical Demographics and Management

Chapter 1
Risk Factors of Hepatocellular Carcinoma for Precision Personalized Care

Naoto Fujiwara, Po-Hong Liu, Sai Krishna Athuluri-Divakar, Shijia Zhu, and Yujin Hoshida

Introduction

Liver cancer, predominantly hepatocellular carcinoma (HCC) arising in the context of cirrhosis, is the second most lethal cancer worldwide with persistently increasing mortality in Europe, North/South America, and Africa in contrast to the decreasing trend in East Asia [1–3]. Cirrhosis is estimated to cause over 1.2 million deaths (2% of global incidences) in 2013 and increased by 47% since 1990 [4]. Cirrhosis and HCC are the major life-limiting consequences of progressive fibrotic liver diseases mainly caused by viral, i.e., hepatitis B virus (HBV) and hepatitis C virus (HCV), and metabolic, i.e., alcohol abuse and nonalcoholic fatty liver disease (NAFLD), etiologies [5]. In the USA, HCC is the fastest rising cause of cancer-related deaths; HCC mortality rate has been increasing across almost all counties over the past three decades particularly in HCV-infected white men aged 55 to 64 years old and Hispanics affected with NAFLD in the Texas region [6–8]. In a model-based

N. Fujiwara
Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

P.-H. Liu · S. K. Athuluri-Divakar · S. Zhu · Y. Hoshida (✉)
Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA
e-mail: Yujin.Hoshida@UTSouthwestern.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_1

simulation forecasting until 2030, HCC incidence rate will continue increasing in the 1950–1959 birth cohorts, Hispanic men, and black women [9].

HCC is highly refractory to therapeutic interventions. Even after surgical resection or ablation, 70% of patients experience tumor recurrence within 5 years [10], and once the tumors progress into advanced stage, currently available medical therapies yield only marginal survival benefit and are not cost-effective [11]. Furthermore, the highly complex and heterogeneous genetic aberrations in HCC tumors hamper identification of therapeutic strategies despite the emerging breadth of molecular targeted anticancer agents [12]. Thus, it will be a rational approach to consider preventing HCC development and progression in patients at risk rather than treating advanced-stage disease with limited health benefit. However, despite the clinical unequivocal predisposing factors for liver disease progression toward cirrhosis and HCC, cancer prevention in this setting remains a daunting task as evidenced by the still dismal HCC prognosis with 5-year survival rate below 15% [13]. In this chapter, we overview limitations of the currently available measures of HCC prevention and opportunities to develop individual cancer risk-based tailored preventive strategies in the era of precision medicine.

Overview of HCC Prevention Strategies

Cancer prevention encompasses a wide variety of medical interventions. Primary prevention focuses on preventing exposure to cancer-predisposing factors or eliminating them at an early stage by vaccination, lifestyle modification, or environmental interventions in an etiology-specific manner (Fig. 1.1). Secondary or tertiary prevention covers early detection and chemoprevention of HCC occurrence or recurrence, respectively, in patients already exposed to etiological agents [14]. Tertiary prevention after radical HCC treatment aims to reduce either recurrence arisen from dissemination of residual tumor cells (disseminative recurrence) or *de novo* carcinogenesis in remnant fibrotic/cirrhotic livers (*de novo* recurrence).

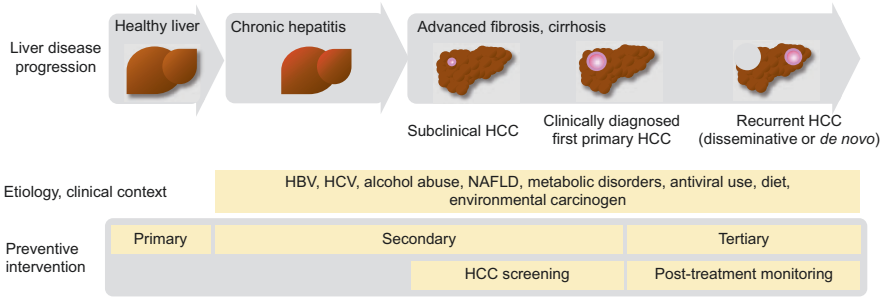

Fig. 1.1 Natural history of HCC development in progressive fibrotic liver diseases and preventive interventions. HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease

Regular HCC screening twice a year is recommended in the current HCC practice guidance as a measure of secondary prevention [15]. However, its implementation in clinical practice is far from satisfactory, as detailed in the next section.

Regular HCC Screening

Screening is a vital component of cancer prevention. Current practice guidelines recommend regular HCC screening (or interchangeably, surveillance) by biannual ultrasound with or without α-fetoprotein (AFP) in clinically identifiable population with HCC risk exceeding a certain threshold [16]. A series of cohort studies and model-based simulation indicate that HCC screening is cost-effective and associated with improved early tumor detection, curative treatment rates, and survival, when it is available to more than 34% of patients at risk [17–21]. However, the real-world utilization rate is below 20% due to multiple patient- and provider-related factors [22]. Population-based interventions such as mailed outreach could improve the utilization rate to up to 50% [23]. With the currently available resources, the vast size of the target population is another obstacle given that cirrhosis is estimated to affect 1–2% of the global population and cause over 1.2 million (and 2% of total) deaths in 2013 and increased by 47% since 1990 [4]. The magnitude of HCC risk for emerging populations, i.e., patients with noncirrhotic NAFLD as well as after HCV cure, is yet to be determined, and screening strategies for these populations have not been established [22]. These issues highlight the limitation of the current one-size-fits-all approach, which assumes uniform HCC risk across all patients and results in often harmful over- or under-estimation of HCC risk for each individual patient [24, 25]. Thus, prediction of individual HCC risk is critical to implementing effective and feasible HCC screening strategy (Fig. 1.2).

Clinical Scores to Predict HCC Risk

Combination of readily available clinical symptoms and laboratory variables has been evaluated to develop HCC risk-predictive scores, although their performance is somewhat limited and yet to be adopted in clinical practice (Table 1.1) [22]. Semi-quantitative histological fibrosis stage has been associated with future HCC risk, although the staging is known to be affected by inter-observer variation [26]. Computational quantification of collagen proportionate area is an objective and more reliable measurement of fibrous tissue amount for estimation of HCC risk, but it is still a liver-biopsy-based method, which is not free from the issue of sampling variability [27–29]. Hemodynamic measurement of portal hypertension, hepatic venous pressure gradient (HVPG), has been associated with HCC risk [30]. Liver stiffness measurement (LSM) by ultrasound- or magnetic resonance imaging (MRI)-based elastography, by presumably capturing fibrotic and inflammatory tissue

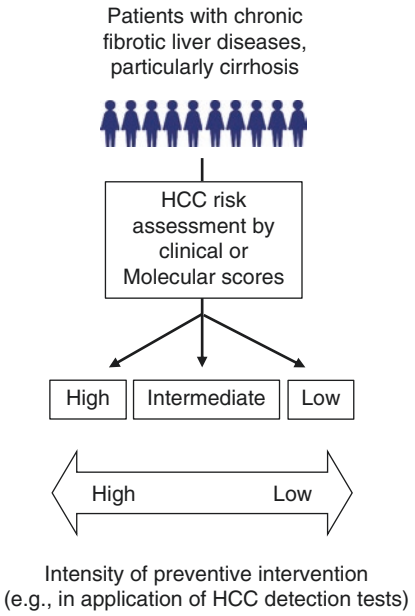

Fig. 1.2 Individual risk-stratified HCC preventive intervention

Table 1.1 Clinical risk scores to predict future HCC development

Risk indicator	Endpoint	Major etiology	Major race/ ethnicity	Variables	Reference
REACH-B	HCC (3/5/10y)	HBV	Asian	Sex, age, ALT, HBeAg, HBV DNA	[83]
CU-HCC	HCC (5y)	HBV	Asian	Age, albumin, bilirubin, HBV DNA, cirrhosis	[84]
LSM-HCC score	HCC (3/5y)	HBV	Asian	LSM, age, albumin, HBV DNA	[85]
Yang et al.	HCC (5/10y)	HBV	Asian	Sex, age, HCC family history, alcohol, ALT, HBeAg, HBV DNA, HBV genotype C	[86]
AGED	HCC (5/10/15/20y)	HBV	Asian	Age, gender, HBeAg, HBV DNA	[87]
Hung et al.	HCC (10y)	HBV	Asian	Age, sex, ALT, previous liver disease, HCC family history, smoking, HBV, HCV	[88]
PAGE-B	HCC (5y)	HBV	White	Age, sex, platelet	[89]
Modified PAGE-B	HCC (5y)	HBV	Asian	Age, sex, platelet, albumin	[90]

Table 1.1 (continued)

Risk indicator	Endpoint	Major etiology	Major race/ ethnicity	Variables	Reference
Sohn et al.	HCC (5y)	HBV	Asian	Age, sex, cirrhosis	[91]
FIB-4	HCC	HBV	Asian	AST, ALT, platelet, age	[92]
GAG-HCC	HCC (5/10y)	HBV	Asian	Age, sex, HBV DNA, core promoter mutations, cirrhosis	[93]
Shin et al.	HCC (5y)	HBV	Asian	LSM, spleen diameter, platelet	[33]
Kim et al.	HCC	HBV	Asian	LSM	[94]
Singal et al.	HCC (3/5y)	HCV	White, black, Hispanic	23 clinical variables	[95]
REVEAL-HCV	HCC (5y)	HCV	Asian	Age, ALT, AST/ALT, HCV RNA, cirrhosis, HCV genotype	[96]
Ganne-Carrié et al.	HCC (3y)	HCV	n.a.	Age, past alcohol abuse, platelet, GGT, SVR	[97]
Nakagomi et al.	HCC	HCV	Asian	LSM	[98]
Lok et al.	HCC (5y)	HCV	White, black, Hispanic	Age, race, platelet, ALP, esophageal varices, smoking	[99]
El-Serag et al.	HCC	HCV	White, black	AFP, ALT, platelet, age	[100]
Huang et al.	HCC	HCV	n.a.	CPA	[28]
Motosugi et al.	HCC	HCV	Asian	LSM by MRE	[31]
Chang et al.	HCC (5y)	HCV after interferon	Asian	Age, sex, platelet, AFP, advanced fibrosis, HCV genotype 1b, SVR	[101]
Ikeda et al.	HCC	HCV after SVR	Asian	Age, AST, platelet before interferon treatment	[102]
$score_{HCC}$	HCC	HCV after SVR	Asian	Age, AFP, platelet, advanced fibrosis	[103]
ALBI	HCC	HCV after SVR	White	Albumin, bilirubin	[104]
Wang et al.	HCC	HCV after SVR	Asian	LSM, advanced fibrosis, diabetes	[34]
ADRESS-HCC	HCC (1y)	HCV, alcohol, NASH/ cryptogenic	White, Hispanic	Age, diabetes, race, etiology, sex, Child-Pugh score	[105]

(continued)

Table 1.1 (continued)

Risk indicator	Endpoint	Major etiology	Major race/ ethnicity	Variables	Reference
Velázquez et al.	HCC (4y)	Alcohol, HCV	n.a.	Age, HCV, prothrombin time, platelet	[106]
VFMAP	HCC (5y)	Nonviral, HCV	Asian	LSM, fast plasma glucose, sex, age, AFP	[107]
Wen et al.	HCC (10y)	General population	Asian	Smoking, alcohol, physical activity, diabetes, AST, ALT, AFP, HBV, HCV	[108]
Singh et al.	HCC, fibrosis (decompensation)	HCV, HBV, alcohol, NASH	n.a.	LSM by TE, MRE	[32]
Konerman et al.	Fibrosis (>2 Ishak score, 1.5/3.5y)	HCV	White	25 clinical variables	[109]
Lens et al.	Fibrosis (F4, 5/7/10y)	HCV	White	Advanced fibrosis, age, AST, GGT, Forns score	[110]
FILI score	Fibrosis after lifestyle intervention (1y)	NASH	n.a.	HbA1c change, platelet, ALT normalization	[111]
ELF score	Fibrosis (F3–4)	HCV, HBV	n.a.	Hyaluronic acid, TIMP1, PIIINP	[112]
Tsochatzis et al.	Fibrosis (decompensation)	Alcohol, HCV	n.a.	CPA	[27]

HCC hepatocellular carcinoma, *HBV* hepatitis B virus, *LSM* liver stiffness measurement, *ALT* alanine aminotransferase, *HCV* hepatitis C virus, *AST* aspartate aminotransferase, *GGT*, γ-glutamyltransferase, *SVR* sustained virologic response, *ALP* alkaline phosphatase, *AFP* α-fetoprotein, *CPA* collagen proportionate area, *MRE* magnetic resonance elastography, *NASH* nonalcoholic steatohepatitis, *VFMAP* virtual touch quantification, fast plasma glucose, sex, age, and AFP, *TE* transient elastography, *FILI* fibrosis improvement after lifestyle interventions, *ELF* enhanced liver fibrosis, *TIMP1* tissue inhibitor of metalloproteinase-1, *PIIINP* propeptide of type III procollagen

contents, has been associated with an increased risk of HCC mostly in viral hepatitis, including cured HCV infection [31–34]. Smoking has been associated with increased HCC risk (relative risk [RR], 1.51) in a meta-analysis of 38 cohort and 58 case-control studies [35] and has been incorporated in several HCC risk scores. The population attributable fraction (PAF) of smoking for HCC was 9% in the USA. [36] Passive smoking was also associated with HCC development (odds ratio [OR] at home, 4.86; OR at work, 2.44) [37]. Association of metabolic HCC risk factors is augmented by smoking (interaction $p = 0.004$) [38]. Alcohol exposure may also enhance risk, as suggested by characteristic somatic DNA aberrations [12].

Molecular Biomarkers to Predict HCC Risk

Molecular biomarkers of HCC risk have been actively explored. Some of them were combined with clinical prognostic factors to develop integrative HCC risk scores to complement clinical scoring systems to refine HCC risk prediction (Table 1.2).

Table 1.2 Molecular biomarkers related to future HCC

Type of molecular information	Molecular feature	HCC endpoint	Major etiology	Major race/ ethnicity	Combined clinical variables	Reference
SNP	*1p36.22* (rs17401966, A >G)	Presence	HBV	Asian	n.a.	[50]
	STAT4 (rs7574865, G >T)	Presence	HBV	Asian	n.a.	[42]
	HLA-DQB1/ HLA-DBA2 (rs9275319, A >G)	Presence	HBV	Asian	n.a.	[42, 113, 114]
	EGF 61AG (rs4444903, A >G)	Development	HCV	White, black	Age, sex, smoking history, ALP, platelet	[115–117]
	IFNL3 (rs8099917, T >G)	Development	HCV	Asian	n.a.	[118–120]
	MICA (rs2596542, G >A)	Presence	HCV	Asian	n.a.	[43, 121, 122]
	DEPDC5 (rs1012068, T >G)	Presence	HCV	Asian	n.a.	[44]
	TLL1 (rs17047200, A >T)	Development	HCV after SVR	Asian	Age, albumin, AFP after SVR	[50]
	PNPLA3 I148M (rs738409, C >G)	Development	Alcohol, HCV	White	Age, sex, BMI	[123–126]
	TM6SF2 E167K (rs58542926, C >T)	Development	Alcohol	White	n.a.	[126–128]
	MBOAT7 (rs641738, C >T)	Presence	NAFLD	White	n.a.	[49]
	MPO -463AG (rs2333227, A >G)	Development	HCV	White	n.a.	[40]
	HFE C282Y (rs1800562, G >A)	Development	Alcohol, HCV	White	n.a.	[129]

(continued)

Table 1.2 (continued)

Type of molecular information	Molecular feature	HCC endpoint	Major etiology	Major race/ ethnicity	Combined clinical variables	Reference
Tissue transcriptome	186/32-gene signature	Development, recurrence	HCV, HBV, Alcohol, NASH	Asian + n.a./ white/ white/ Asian	AFP, vascular invasion, bilirubin, platelet, Child-Pugh class, AJCC stage	[10, 130, 131]
	HIR gene signature	Recurrence (>2 year)	HBV	Asian	n.a.	[132]
	ELS signature	Recurrence (>2 year)	HCV	Asian	n.a.	[133]
	Activated HSC gene signature	Recurrence	HBV	Asian	n.a.	[134]
	HSC signature	Recurrence	HCV, HBV	White/ Asian	Bilirubin, platelet	[135]

SNP single nucleotide polymorphism; *HSC* hepatic stellate cell, *HIR* hepatic injury and regeneration, *ELS* ectopic lymphoid-like structures, *IGF* insulin-like growth factor, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *HCC* hepatocellular carcinoma, *NAFLD* nonalcoholic fatty liver disease, *HCV* hepatitis C virus, *SVR* sustained virologic response, *NASH* nonalcoholic steatohepatitis, *HBV* hepatitis B virus, *BMI* body mass index, *AFP* α-fetoprotein, *ALP* alkaline phosphatase

Several germline single nucleotide polymorphisms (SNPs) have been identified as indicators of elevated HCC risk with odds ratios of around 1.5 in prospective and retrospective cohorts: *EGF* (in HBV- or HCV-infected patients); *MPO*, *DEPDC5*, and *MICA* (in HCV-infected patients); region in 1p36.22, *STAT4*, and *HLA-DQ* (in HBV-infected patients); and *PNPLA3* and *TM6SF2* (in alcoholic liver disease and NAFLD patients) [39–47]. Shorter telomeres and germline mutations in *TERT* gene were observed in NAFLD-related HCC patients [48]. A SNP in *MBOAT7* gene was linked to HCC in noncirrhotic NAFLD patients [49]. A recent genome-wide association study identified a SNP in *TLL1* gene associated with HCC risk after HCV cure [50]. A 7-gene SNP panel (Cirrhosis Risk Score) was associated with fibrosis progression in HCV-infected individuals [51]. Liver tissue-derived transcriptome signatures have been associated with HCC risk. For example, a 32-gene signature in fibrotic liver has been validated as a pan-etiology HCC risk indicator in patients with chronic hepatitis B/C, alcohol abuse, and nonalcoholic steatohepatitis (NASH) [10]. Abundance of serum/plasma proteins such as insulin-like growth factor 1 (IGF1) and osteopontin (OPN/*SPP1*) has also been associated with HCC risk in cirrhosis [52, 53]. The N-glycosylation pattern of total serum protein (GlycoHCCRiskScore) has identified a subset of compensated cirrhosis patients at HCC risk [54]. Body fluid (e.g., blood, urine)-based biomarkers will enable less

invasive and more flexible prognostic prediction given the decreasing utilization of liver biopsies in clinical practice, although tissue acquisition will help ensure their relevance to liver disease at least during the process of establishing such assays. Scientifically rigorous biomarker validation following the predefined phases of biomarker development will help ensure clinical validity of the biomarkers [55]. These biomarkers are promising candidates for clinical application, although assay development and implementation, regulatory approval, and reimbursement are challenging obstacles [56].

HCC Detection Modalities and Biomarkers for Regular HCC Screening

Abdominal ultrasound and serum AFP have been widely used as the main HCC screening modalities. The suggested minimal sensitivity for an HCC screening test to be cost-effective is 42% assuming a screening access rate of 34% [21]. The sensitivity of ultrasound and AFP for detection of early-stage HCC tumor exceeds the threshold (approximately 60%), although it is still considered suboptimal [57]. Operator dependency and patient-related factors such as obesity are the major sources of variation in ultrasound sensitivity, which can be as low as 32% [58–60]. Serum AFP levels can nonspecifically rise due to chronic hepatitis-related liver regeneration, which raises concern about its clinical utility as a screening modality [61]. New serum or plasma biomarkers have been explored as possible replacements for AFP, and some of them are awaiting larger clinical validation for further development and deployment (Table 1.3). Integrative scores combining serum biomarkers with clinical variables have been proposed to improve diagnostic performance [62, 63]. In addition, identification of specific clinical contexts (e.g., HCV cirrhosis with normal serum alanine aminotransferase [ALT] level) has been suggested as a strategy to achieve improved performance of AFP [64]. An integrative score combining fucosylated kininogen, AFP, and clinical variables yielded highly accurate detection of early-stage HCC [65]. Circulating cell-free DNA and its epigenomic alterations have also shown encouraging results to detect HCC in both experimental studies and clinical trials [66, 67].

Computed tomography (CT) and MRI may serve as alternatives to ultrasound with better performance and are free from interoperator variability. Indeed, CT and MRI can double the lesion-based sensitivity for small HCC tumors (up to 86%), although the high costs and irradiation (for CT) preclude their use as practical widespread options for HCC screening [68–70]. Abbreviated contrast-enhanced MRI (AMRI) has been developed as an option that is specifically designed for regular HCC screening at half the cost of a full MRI while maintaining a high sensitivity (81%) and specificity (96%) [71].

Table 1.3 Clinical and experimental biomarkers to diagnose HCC

Biomarker	Major etiology	Major race/ethnicity	Sensitivity	Specificity	AUROC	Reference
Biomarker in clinical use						
AFP	n.a.	White, Asian	4–71%	29–100%	n.a.	[136, 137]
AFP-L3	HCV	Asian	21–49%	93–100%	0.69	[137]
DCP	n.a.	Asian	28–89%	68–100%	0.86	[138]
Integrative score						
GALAD model∗	Alcohol, HCV, HBV	n.a.	82%	82%	0.91	[63, 139, 140]
Doylestown algorithm (DA)	HBV/HCVC + HCV/HBV/HCV∗	n.a.	45%	Fixed to 95%	0.81	[62, 141]
Experimental biomarker						
GPC3∗	HBV, HCV	n.a.	55%	97%	n.a.	[142]
microRNA panel∗	HBV	Asian	83–86%	77–84%	0.89	[143]
DKK1∗	HBV	Asian	74%	91%	0.91	[144]
MDK	HBV, NASH	Asian	86%	90%	n.a.	[145, 146]
Annexin A2∗	HBV	Asian	86%	74%	0.80	[147]
GlycoHCCTest	HBV	Asian	57%	88%	0.81	[148]
Osteopontin∗	n.a.	n.a.	49%	72%	n.a.	[149]
GP73∗	HCV	White	62%	88%	0.77	[150]
GlycoHCCRiskTest	HCV	n.a.	n.a.	n.a.	0.73	[54]
DA plus (DA + kininogen)∗	HCV	n.a.	86%	Fixed to 95%	0.97	[65]
Plasma methylated DNA	HCV, alcohol, NAFLD	n.a.	95%	92%	0.96	[67]

∗The performance is for early-stage HCC detection. *HCC* hepatocellular carcinoma, *AUROC* area under the receiver operating characteristic curve, *AFP* α-fetoprotein, *HCV* hepatitis C virus, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-gamma-carboxy prothrombin, *GALAD* gender, age, AFP-L3, AFP, des-carboxy prothrombin, *HBV* hepatitis B virus, *GPC3* glypican 3, *BCLC* Barcelona Clinic Liver Cancer, *DKK1* Dickkopf-1, *MDK* midkine, *AJCC* American Joint Committee on Cancer, *GP73* Golgi protein-73, *UNOS* United Network for Organ Sharing, *NAFLD* nonalcoholic fatty liver disease

HCC Biomarker Development

Despite the numerous HCC biomarker candidates in literature, virtually none of them has been translated into clinic to date. This is primarily due to the highly demanding process of clinical translation: (i) biomarker discovery and validation, (ii) assay development, (iii) analytical validation, (iv) clinical utility validation in

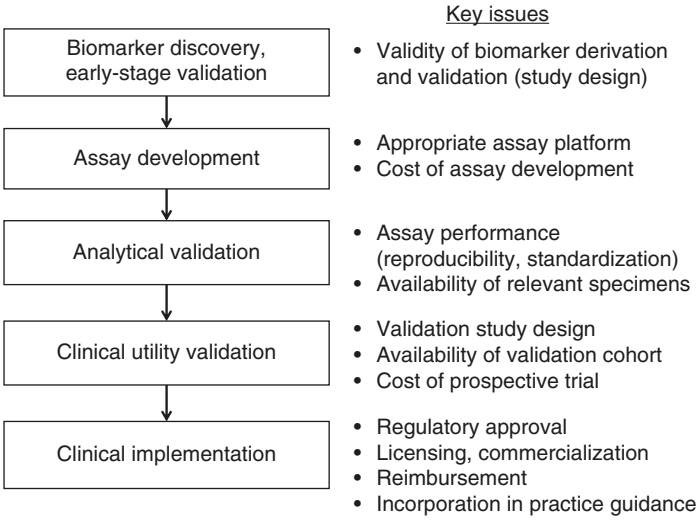

Fig. 1.3 Steps to clinically translate HCC biomarker

prospective trial, and (v) clinical implementation for regulatory approval, commercialization, reimbursement, and incorporation in practice guidance (Fig. 1.3) [56]. It is indeed practically infeasible to follow the costly and lengthy process for every single biomarker candidate. To address the challenge, a prospective-specimen-collection, retrospective-blinded-evaluation (PRoBE) design has been proposed for the evaluation of diagnostic, prognostic, and screening biomarkers [55]. In this framework, biospecimens and relevant clinical annotations are prospectively collected from a cohort of patients, representing the target population of biomarker application (e.g., cirrhosis patients at risk of HCC development) without intension of assessing any specific biomarker. Prospective and longitudinal follow-up of the cohort eventually reveals clinical outcomes of interest (e.g., HCC development), and case and control patients are determined. At this stage, a candidate biomarker can be blindly evaluated in randomly chosen case and control patients using the stored biospecimens without concern about potential biases frequently seen in retrospective studies (Fig. 1.4). This strategy avoids replicating the costly, lengthy, and laborious prospective cohort generation, the major bottleneck of clinical biomarker development, and will create invaluable resource to facilitate clinical translation of promising biomarker candidates. In HCC, a few resources have been established with maturing prospective clinical follow-up information in several thousand patients, e.g., US National Cancer Institute's Early Detection Research Network (EDRN) for HCC [72] and Texas Hepatocellular Carcinoma Consortium (THCCC) [73]. EDRN adopts an approach of biomarker development consisting of five phases: preclinical exploratory studies (phase 1); clinical assay development for clinical disease, analyzing specimens collected from case patients at the time of observing endpoint of interest (phase 2); retrospective longitudinal repository studies, using specimens collected from case patients before observing endpoint of interest (phase 3);

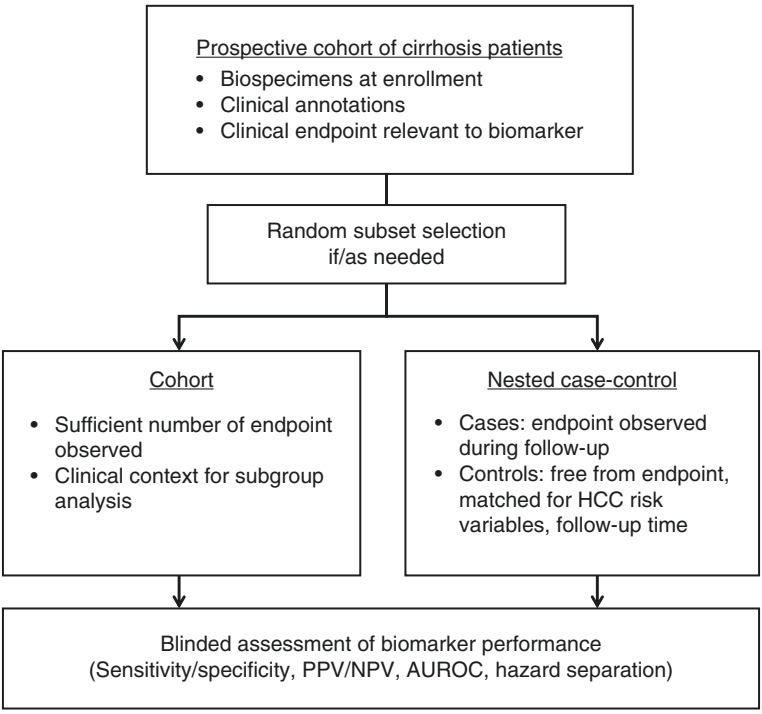

Fig. 1.4 The PRoBE design for HCC biomarker evaluation

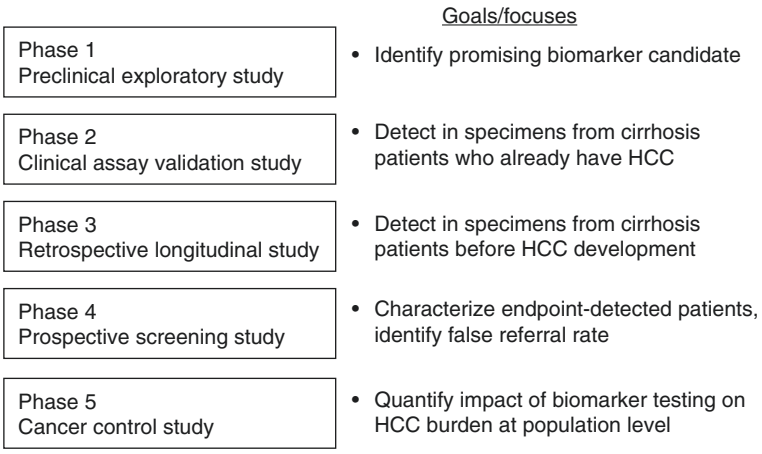

Fig. 1.5 Phases of HCC biomarker development study

prospective screening studies (phase 4); and cancer control studies (phase 5) (Fig. 1.5) [74]. With the resources such as EDRN and THCCC in line with the PRoBE design principle, one can skip phase 2 and directly move to the pivotal phase 3 study, following discovery and early-stage validation of promising biomarker

candidates. Simulation-based cost-effectiveness analysis is a useful tool to quantitatively estimate population-level net benefit of clinically implementing a biomarker in actual clinical setting (i.e., the goal of phase 5 study), where real-world application rate can also be modeled [75]. These resources and framework will significantly facilitate clinical translation of HCC risk-predictive and detection biomarkers.

Individual Risk-Based Tailored HCC Screening

The heterogeneous individual HCC risk among the patients captured by clinical and molecular scores will enable rational allocation of the limited HCC screening resources to the high-risk patients who need screening the most and avoid ineffective and unnecessary distribution of the demanding screening efforts to low-risk individuals. The currently recommended HCC screening interval of 6 months was determined based on estimated tumor volume doubling time [76, 77]. Uniformly longer or shorter intervals did not improve HCC detection [78, 79]. However, given that high-risk subjects likely develop HCC at a high frequency and in a multicentric manner, altering HCC screening intensity according to estimated individual HCC risk may enable more efficient early tumor detection (Fig. 1.2) [24]. Such a personalized risk-based cancer screening strategy has been successfully implemented in other tumor types such as colorectal and breast cancers [80, 81]. In addition, education programs targeting high-risk communities with specific HCC risks based on etiology, for example, African-born immigrants in New York City with a high prevalence of HBV infection, may efficiently improve uptake of high-risk individuals to HCC screening [82].

The net benefit of HCC screening is determined as a function of multiple factors, including screening interval, performance of screening modalities, HCC incidence in the target population, and screening access rate, which has been evaluated by model-based cost-effectiveness analysis. A recent comprehensive assessment of individual risk-based tailored HCC screening strategies revealed superior cost-effectiveness of personalized screening compared to the currently recommended uniform biannual screening of all patients [75]. For instance, exclusive screening of high-risk subjects using AMRI is a robustly cost-effective strategy. More frequent screening, i.e., four times per year, is cost-effective when annual HCC incidence is greater than 3%. Although these results need to be clinically verified, testing of such strategies is now technically feasible with the HCC risk tests, and new screening modalities are already available in the clinical setting.

Conclusions

Clinical evaluation and implementation of HCC preventive strategies, including HCC screening, will not be successful nor feasible without individual risk-based tailored approaches. Diversity in HCC incidence according to etiology, patient race/ethnicity, and clinical context needs to be considered in assessing clinical utility and

real-world effectiveness of preventive interventions. The precision medicine approaches rely on molecular information derived from biospecimens. Although liver tissue is the most reliable source to measure pathogenic molecular dysregulation, transition to less invasive types of biospecimen will help widen its applicability. Sampling bias and robustness in molecular readout should also be determined in preclinical and clinical studies. Once these issues are resolved and the preventive strategies are clinically implemented, the tailored approach will enable more cost-effective and precise preventive intervention in the clinical care of patients at HCC risk, which will substantially improve the dismal prognosis of HCC.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. https://doi.org/10.3322/caac.21262.
2. Bertuccio P, Turati F, Carioli G, Rodriguez T, La Vecchia C, Malvezzi M, et al. Global trends and predictions in hepatocellular carcinoma mortality. J Hepatol. 2017;67(2):302–9. https://doi.org/10.1016/j.jhep.2017.03.011.
3. Yang JD, Mohamed EA, Aziz AO, Shousha HI, Hashem MB, Nabeel MM, et al. Characteristics, management, and outcomes of patients with hepatocellular carcinoma in Africa: a multicountry observational study from the Africa Liver Cancer Consortium. Lancet Gastroenterol Hepatol. 2017;2(2):103–11. https://doi.org/10.1016/S2468-1253(16)30161-3.
4. Mortality GBD. Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117–71. https://doi.org/10.1016/S0140-6736(14)61682-2.
5. Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev. 2017;121:27–42. https://doi.org/10.1016/j.addr.2017.05.007.
6. Mokdad AH, Dwyer-Lindgren L, Fitzmaurice C, Stubbs RW, Bertozzi-Villa A, Morozoff C, et al. Trends and Patterns of Disparities in Cancer Mortality Among US Counties, 1980-2014. JAMA. 2017;317(4):388–406. https://doi.org/10.1001/jama.2016.20324.
7. White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of hepatocellular carcinoma in all 50 United States, from 2000 through 2012. Gastroenterology. 2017;152(4):812–20 e5. https://doi.org/10.1053/j.gastro.2016.11.020.
8. Ryerson AB, Eheman CR, Altekruse SF, Ward JW, Jemal A, Sherman RL, et al. Annual report to the Nation on the status of cancer, 1975-2012, featuring the increasing incidence of liver cancer. Cancer. 2016;122(9):1312–37. https://doi.org/10.1002/cncr.29936.
9. Petrick JL, Kelly SP, Altekruse SF, McGlynn KA, Rosenberg PS. Future of hepatocellular carcinoma incidence in the United States forecast through 2030. J Clin Oncol. 2016;34(15):1787–94. https://doi.org/10.1200/JCO.2015.64.7412.
10. Nakagawa S, Wei L, Song WM, Higashi T, Ghoshal S, Kim RS, et al. Molecular liver cancer prevention in cirrhosis by organ transcriptome analysis and lysophosphatidic acid pathway inhibition. Cancer Cell. 2016;30(6):879–90. https://doi.org/10.1016/j.ccell.2016.11.004.
11. Parikh ND, Singal AG, Hutton DW. Cost effectiveness of regorafenib as second-line therapy for patients with advanced hepatocellular carcinoma. Cancer. 2017;123(19):3725–31. https://doi.org/10.1002/cncr.30863.
12. Cancer Genome Atlas Research Network. Electronic address wbe, Cancer Genome Atlas Research N. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;169(7):1327–41 e23. https://doi.org/10.1016/j.cell.2017.05.046.

13. Goossens N, Sun X, Hoshida Y. Molecular classification of hepatocellular carcinoma: potential therapeutic implications. Hepat Oncol. 2015;2(4):371–9. https://doi.org/10.2217/hep.15.26.
14. Hoshida Y, Fuchs BC, Tanabe KK. Prevention of hepatocellular carcinoma: potential targets, experimental models, and clinical challenges. Curr Cancer Drug Targets. 2012;12(9):1129–59.
15. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the study of liver diseases. Hepatology. 2018;68(2):723–50. https://doi.org/10.1002/hep.29913.
16. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67(1):358–80. https://doi.org/10.1002/hep.29086.
17. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. https://doi.org/10.1371/journal.pmed.1001624.
18. Mittal S, Kanwal F, Ying J, Chung R, Sada YH, Temple S, et al. Effectiveness of surveillance for hepatocellular carcinoma in clinical practice: a United States cohort. J Hepatol. 2016;65(6):1148–54. https://doi.org/10.1016/j.jhep.2016.07.025.
19. Singal AG, Mittal S, Yerokun OA, Ahn C, Marrero JA, Yopp AC, et al. Hepatocellular carcinoma screening associated with early tumor detection and improved survival among patients with cirrhosis in the US. Am J Med. 2017. https://doi.org/10.1016/j.amjmed.2017.01.021.
20. Cadier B, Bulsei J, Nahon P, Seror O, Laurent A, Rosa I, et al. Early detection and curative treatment of hepatocellular carcinoma: a cost-effectiveness analysis in France and in the United States. Hepatology. 2017;65(4):1237–48. https://doi.org/10.1002/hep.28961.
21. Mourad A, Deuffic-Burban S, Ganne-Carrie N, Renaut-Vantroys T, Rosa I, Bouvier AM, et al. Hepatocellular carcinoma screening in patients with compensated hepatitis C virus (HCV)-related cirrhosis aware of their HCV status improves survival: a modeling approach. Hepatology. 2014;59(4):1471–81. https://doi.org/10.1002/hep.26944.
22. Singal AG, El-Serag HB. Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. Clin Gastroenterol Hepatol. 2015;13(12):2140–51. https://doi.org/10.1016/j.cgh.2015.08.014.
23. Singal AG, Tiro JA, Marrero JA, McCallister K, Mejias C, Adamson B, et al. Mailed outreach program increases ultrasound screening of patients with cirrhosis for hepatocellular carcinoma. Gastroenterology. 2017;152(3):608–15 e4. https://doi.org/10.1053/j.gastro.2016.10.042.
24. Goossens N, Bian CB, Hoshida Y. Tailored algorithms for hepatocellular carcinoma surveillance: is one-size-fits-all strategy outdated? Curr Hepatol Rep. 2017;16(1):64–71. https://doi.org/10.1007/s11901-017-0336-z.
25. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, Parikh ND, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. Hepatology. 2017;65(4):1196–205. https://doi.org/10.1002/hep.28895.
26. Germani G, Hytiroglou P, Fotiadu A, Burroughs AK, Dhillon AP. Assessment of fibrosis and cirrhosis in liver biopsies: an update. Semin Liver Dis. 2011;31(1):82–90. https://doi.org/10.1055/s-0031-1272836.
27. Tsochatzis E, Bruno S, Isgro G, Hall A, Theocharidou E, Manousou P, et al. Collagen proportionate area is superior to other histological methods for sub-classifying cirrhosis and determining prognosis. J Hepatol. 2014;60(5):948–54. https://doi.org/10.1016/j.jhep.2013.12.023.
28. Huang Y, de Boer WB, Adams LA, MacQuillan G, Bulsara MK, Jeffrey GP. Image analysis of liver biopsy samples measures fibrosis and predicts clinical outcome. J Hepatol. 2014;61(1):22–7. https://doi.org/10.1016/j.jhep.2014.02.031.
29. Wang TH, Chen TC, Teng X, Liang KH, Yeh CT. Automated biphasic morphological assessment of hepatitis B-related liver fibrosis using second harmonic generation microscopy. Sci Rep. 2015;5:12962. https://doi.org/10.1038/srep12962.

30. Suk KT, Kim EJ, Kim DJ, Kim HS, Bang CS, Park TY, et al. Prognostic significance of hemodynamic and clinical stages in the prediction of hepatocellular carcinoma. J Clin Gastroenterol. 2017;51(3):285–93. https://doi.org/10.1097/MCG.0000000000000671.
31. Motosugi U, Ichikawa T, Koshiishi T, Sano K, Morisaka H, Ichikawa S, et al. Liver stiffness measured by magnetic resonance elastography as a risk factor for hepatocellular carcinoma: a preliminary case-control study. Eur Radiol. 2013;23(1):156–62. https://doi.org/10.1007/s00330-012-2571-6.
32. Singh S, Fujii LL, Murad MH, Wang Z, Asrani SK, Ehman RL, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2013;11(12):1573–84 e1-2.; quiz e88-9. https://doi.org/10.1016/j.cgh.2013.07.034.
33. Shin SH, Kim SU, Park JY, Kim DY, Ahn SH, Han KH, et al. Liver stiffness-based model for prediction of hepatocellular carcinoma in chronic hepatitis B virus infection: comparison with histological fibrosis. Liver Int. 2015;35(3):1054–62. https://doi.org/10.1111/liv.12621.
34. Wang JH, Yen YH, Yao CC, Hung CH, Chen CH, Hu TH, et al. Liver stiffness-based score in hepatoma risk assessment for chronic hepatitis C patients after successful antiviral therapy. Liver Int. 2016;36(12):1793–9. https://doi.org/10.1111/liv.13179.
35. Lee YC, Cohet C, Yang YC, Stayner L, Hashibe M, Straif K. Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. Int J Epidemiol. 2009;38(6):1497–511. https://doi.org/10.1093/ije/dyp280.
36. Makarova-Rusher OV, Altekruse SF, McNeel TS, Ulahannan S, Duffy AG, Graubard BI, et al. Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. Cancer. 2016;122(11):1757–65. https://doi.org/10.1002/cncr.29971.
37. Niu J, Lin Y, Guo Z, Niu M, Su C. The epidemiological investigation on the risk factors of hepatocellular carcinoma: a case-control study in Southeast China. Medicine. 2016;95(6):e2758. https://doi.org/10.1097/MD.0000000000002758.
38. Yu MW, Lin CL, Liu CJ, Yang SH, Tseng YL, Wu CF. Influence of metabolic risk factors on risk of hepatocellular carcinoma and liver-related death in men with chronic hepatitis B: a large cohort study. Gastroenterology. 2017. https://doi.org/10.1053/j.gastro.2017.07.001.
39. Zhong JH, You XM, Gong WF, Ma L, Zhang Y, Mo QG, et al. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One. 2012;7(3):e32159. https://doi.org/10.1371/journal.pone.0032159.
40. Nahon P, Sutton A, Rufat P, Charnaux N, Mansouri A, Moreau R, et al. A variant in myeloperoxidase promoter hastens the emergence of hepatocellular carcinoma in patients with HCV-related cirrhosis. J Hepatol. 2012;56(2):426–32. https://doi.org/10.1016/j.jhep.2011.08.010.
41. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, Jia W, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet. 2010;42(9):755–8. https://doi.org/10.1038/ng.638.
42. Jiang DK, Sun J, Cao G, Liu Y, Lin D, Gao YZ, et al. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. Nat Genet. 2013;45(1):72–5. https://doi.org/10.1038/ng.2483.
43. Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet. 2011;43(5):455–8. https://doi.org/10.1038/ng.809.
44. Miki D, Ochi H, Hayes CN, Abe H, Yoshima T, Aikata H, et al. Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. Nat Genet. 2011;43(8):797–800. https://doi.org/10.1038/ng.876.
45. Huang CF, Huang CI, Yeh ML, Wang SC, Chen KY, Ko YM, et al. Diversity of the association of serum levels and genetic variants of MHC class I polypeptide-related chain A with liver fibrosis in chronic hepatitis C. Oncotarget. 2017;8(20):32618–25. https://doi.org/10.18632/oncotarget.15941.
46. Zhang S, Qiao K, Trieu C, Huo Z, Dai Q, Du Y, et al. Genetic polymorphism of epidermal growth factor rs4444903 influences susceptibility to HCV-related liver cirrhosis and hepatocellular carcinoma in a Chinese han population. Clin Lab. 2017;63(4):845–50. https://doi.org/10.7754/Clin.Lab.2016.161203.

47. Trepo E, Romeo S, Zucman-Rossi J, Nahon P. PNPLA3 gene in liver diseases. J Hepatol. 2016;65(2):399–412. https://doi.org/10.1016/j.jhep.2016.03.011.
48. Donati B, Pietrelli A, Pingitore P, Dongiovanni P, Caddeo A, Walker L, et al. Telomerase reverse transcriptase germline mutations and hepatocellular carcinoma in patients with nonalcoholic fatty liver disease. Cancer Med. 2017;6(8):1930–40. https://doi.org/10.1002/cam4.1078.
49. Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, Miele L, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. Sci Rep. 2017;7(1):4492. https://doi.org/10.1038/s41598-017-04991-0.
50. Matsuura K, Sawai H, Ikeo K, Ogawa S, Iio E, Isogawa M, et al. Genome-wide association study identifies TLL1 variant associated with development of hepatocellular carcinoma after eradication of hepatitis C virus infection. Gastroenterology. 2017;152(6):1383–94. https://doi.org/10.1053/j.gastro.2017.01.041.
51. Besheer T, El-Bendary M, Elalfy H, Abd El-Maksoud M, Salah M, Zalata K, et al. Prediction of fibrosis progression rate in patients with chronic hepatitis C genotype 4: role of cirrhosis risk score and host factors. J Interf Cytokine Res. 2017;37(3):97–102. https://doi.org/10.1089/jir.2016.0111.
52. Duarte-Salles T, Misra S, Stepien M, Plymoth A, Muller D, Overvad K, et al. Circulating osteopontin and prediction of hepatocellular carcinoma development in a large European population. Cancer Prev Res (Phila). 2016;9(9):758–65. https://doi.org/10.1158/1940-6207.CAPR-15-0434.
53. Mazziotti G, Sorvillo F, Morisco F, Carbone A, Rotondi M, Stornaiuolo G, et al. Serum insulin-like growth factor I evaluation as a useful tool for predicting the risk of developing hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a prospective study. Cancer. 2002;95(12):2539–45. https://doi.org/10.1002/cncr.11002.
54. Verhelst X, Vanderschaeghe D, Castera L, Raes T, Geerts A, Francoz C, et al. A glycomics-based test predicts the development of hepatocellular carcinoma in cirrhosis. Clin Cancer Res. 2017;23(11):2750–8. https://doi.org/10.1158/1078-0432.CCR-16-1500.
55. Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. J Natl Cancer Inst. 2008;100(20):1432–8. https://doi.org/10.1093/jnci/djn326.
56. Goossens N, Nakagawa S, Sun X, Hoshida Y. Cancer biomarker discovery and validation. Transl Cancer Res. 2015;4(3):256–69. https://doi.org/10.3978/j.issn.2218-676X.2015.06.04.
57. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30(1):37–47. https://doi.org/10.1111/j.1365-2036.2009.04014.x.
58. Singal AG, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. Cancer Epidemiol Biomark Prev. 2012;21(5):793–9. https://doi.org/10.1158/1055-9965.EPI-11-1005.
59. Del Poggio P, Olmi S, Ciccarese F, Di Marco M, Rapaccini GL, Benvegnu L, et al. Factors that affect efficacy of ultrasound surveillance for early stage hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014;12(11):1927–33 e2. https://doi.org/10.1016/j.cgh.2014.02.025.
60. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. Aliment Pharmacol Ther. 2017;45(1):169–77. https://doi.org/10.1111/apt.13841.
61. Song PP, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, Kokudo N, et al. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. World J Gastroenterol. 2016;22(1):262–74. https://doi.org/10.3748/wjg.v22.i1.262.
62. Wang M, Devarajan K, Singal AG, Marrero JA, Dai J, Feng Z, et al. The doylestown algorithm: a test to improve the performance of AFP in the detection of hepatocellular carcinoma. Cancer Prev Res (Phila). 2016;9(2):172–9. https://doi.org/10.1158/1940-6207.CAPR-15-0186.
63. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. Cancer Epidemiol Biomark Prev. 2014;23(1):144–53. https://doi.org/10.1158/1055-9965.EPI-13-0870.

64. Yang JD, Dai J, Singal AG, Gopal P, Addissie BD, Nguyen MH, et al. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. Cancer Epidemiol Biomark Prev. 2017;26(7):1085–92. https://doi.org/10.1158/1055-9965.EPI-16-0747.
65. Wang M, Sanda M, Comunale MA, Herrera H, Swindell C, Kono Y, et al. Changes in the glycosylation of kininogen and the development of a kininogen-based algorithm for the early detection of HCC. Cancer Epidemiol Biomark Prev. 2017;26(5):795–803. https://doi.org/10.1158/1055-9965.EPI-16-0974.
66. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16(11):1155–61. https://doi.org/10.1038/nmat4997.
67. Kisiel JB, Dukek BA, Kanipakam R, Ghoz HM, Yab TC, Berger CK, et al. hepatocellular carcinoma detection by plasma methylated DNA: discovery, phase I pilot, and phase II clinical validation. Hepatology. 2018. https://doi.org/10.1002/hep.30244.
68. Yu NC, Chaudhari V, Raman SS, Lassman C, Tong MJ, Busuttil RW, et al. CT and MRI improve detection of hepatocellular carcinoma, compared with ultrasound alone, in patients with cirrhosis. Clin Gastroenterol Hepatol. 2011;9(2):161–7. https://doi.org/10.1016/j.cgh.2010.09.017.
69. Pocha C, Dieperink E, McMaken KA, Knott A, Thuras P, Ho SB. Surveillance for hepatocellular cancer with ultrasonography vs. computed tomography -- a randomised study. Aliment Pharmacol Ther. 2013;38(3):303–12. https://doi.org/10.1111/apt.12370.
70. Kim SY, An J, Lim Y-S, Han S, Lee J-Y, Byun JH, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. JAMA oncology. 2016.
71. Besa C, Lewis S, Pandharipande PV, Chhatwal J, Kamath A, Cooper N, et al. Hepatocellular carcinoma detection: diagnostic performance of a simulated abbreviated MRI protocol combining diffusion-weighted and T1-weighted imaging at the delayed phase post gadoxetic acid. Abdom Radiol. 2016:1–12.
72. Borges KA, Dai J, Parikh ND, Schwartz M, Nguyen MH, Roberts LR, et al. Rationale and design of the Hepatocellular carcinoma early detection strategy study: a multi-center longitudinal initiative of the National Cancer Institute's early detection research network. Contemp Clin Trials. 2019;76:49–54. https://doi.org/10.1016/j.cct.2018.11.008.
73. Feng Z, Marrero JA, Khaderi S, Singal AG, Kanwal F, Loo N, et al. Design of the texas hepatocellular carcinoma consortium cohort study. Am J Gastroenterol. 2019. https://doi.org/10.14309/ajg.0000000000000068.
74. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93(14):1054–61.
75. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, Besa C, et al. Cost-effectiveness of risk score-stratified hepatocellular carcinoma screening in patients with cirrhosis. Clin Transl Gastroenterol. 2017;8(6):e101. https://doi.org/10.1038/ctg.2017.26.
76. Furlan A, Marin D, Agnello F, Di Martino M, Di Marco V, Lagalla R, et al. Hepatocellular carcinoma presenting at contrast-enhanced multi–detector-row computed tomography or gadolinium-enhanced magnetic resonance imaging as a small (≤ 2 cm), indeterminate nodule: growth rate and optimal interval time for imaging follow-up. J Comput Assist Tomogr. 2012;36(1):20–5.
77. Taouli B, Goh JS, Lu Y, Qayyum A, Yeh BM, Merriman RB, et al. Growth rate of hepatocellular carcinoma: evaluation with serial computed tomography or magnetic resonance imaging. J Comput Assist Tomogr. 2005;29(4):425–9.
78. Santi V, Trevisani F, Gramenzi A, Grignaschi A, Mirici-Cappa F, Del Poggio P, et al. Semiannual surveillance is superior to annual surveillance for the detection of early hepatocellular carcinoma and patient survival. J Hepatol. 2010;53(2):291–7.
79. Trinchet JC, Chaffaut C, Bourcier V, Degos F, Henrion J, Fontaine H, et al. Ultrasonographic surveillance of hepatocellular carcinoma in cirrhosis: a randomized trial comparing 3- and 6-month periodicities. Hepatology. 2011;54(6):1987–97. https://doi.org/10.1002/hep.24545.

80. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin. 2008;58(3):130–60.
81. Tice JA, Cummings SR, Smith-Bindman R, Ichikawa L, Barlow WE, Kerlikowske K. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. Ann Intern Med. 2008;148(5):337–47.
82. Bolutayo K, van Manh AL, Cohen N, Ndiaye D, Jandorf L, Perumalswami PV. Reducing liver cancer risk in African-born immigrants through culturally targeted hepatitis B group education programs. J Cancer Educ. 2017. https://doi.org/10.1007/s13187-017-1231-6.
83. Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol. 2011;12(6):568–74. https://doi.org/10.1016/S1470-2045(11)70077-8.
84. Wong VW, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. J Clin Oncol. 2010;28(10):1660–5. https://doi.org/10.1200/JCO.2009.26.2675.
85. Wong GL, Chan HL, Wong CK, Leung C, Chan CY, Ho PP, et al. Liver stiffness-based optimization of hepatocellular carcinoma risk score in patients with chronic hepatitis B. J Hepatol. 2014;60(2):339–45. https://doi.org/10.1016/j.jhep.2013.09.029.
86. Yang HI, Sherman M, Su J, Chen PJ, Liaw YF, Iloeje UH, et al. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Clin Oncol. 2010;28(14):2437–44. https://doi.org/10.1200/JCO.2009.27.4456.
87. Fan C, Li M, Gan Y, Chen T, Sun Y, Lu J, et al. A simple AGED score for risk classification of primary liver cancer: development and validation with long-term prospective HBsAg-positive cohorts in Qidong, China. Gut. 2018. https://doi.org/10.1136/gutjnl-2018-316525.
88. Hung YC, Lin CL, Liu CJ, Hung H, Lin SM, Lee SD, et al. Development of risk scoring system for stratifying population for hepatocellular carcinoma screening. Hepatology. 2015;61(6):1934–44. https://doi.org/10.1002/hep.27610.
89. Papatheodoridis G, Dalekos G, Sypsa V, Yurdaydin C, Buti M, Goulis J, et al. PAGE-B predicts the risk of developing hepatocellular carcinoma in Caucasians with chronic hepatitis B on 5-year antiviral therapy. J Hepatol. 2016;64(4):800–6. https://doi.org/10.1016/j.jhep.2015.11.035.
90. Kim JH, Kim YD, Lee M, Jun BG, Kim TS, Suk KT, et al. Modified PAGE-B score predicts the risk of hepatocellular carcinoma in Asians with chronic hepatitis B on antiviral therapy. J Hepatol. 2018;69(5):1066–73. https://doi.org/10.1016/j.jhep.2018.07.018.
91. Sohn W, Cho JY, Kim JH, Lee JI, Kim HJ, Woo MA, et al. Risk score model for the development of hepatocellular carcinoma in treatment-naive patients receiving oral antiviral treatment for chronic hepatitis B. Clin Mol Hepatol. 2017;23(2):170–8. https://doi.org/10.3350/cmh.2016.0086.
92. Suh B, Park S, Shin DW, Yun JM, Yang HK, Yu SJ, et al. High liver fibrosis index FIB-4 is highly predictive of hepatocellular carcinoma in chronic hepatitis B carriers. Hepatology. 2015;61(4):1261–8. https://doi.org/10.1002/hep.27654.
93. Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol. 2009;50(1):80–8. https://doi.org/10.1016/j.jhep.2008.07.023.
94. Kim MN, Kim SU, Kim BK, Park JY, Kim DY, Ahn SH, et al. Increased risk of hepatocellular carcinoma in chronic hepatitis B patients with transient elastography-defined subclinical cirrhosis. Hepatology. 2015;61(6):1851–9. https://doi.org/10.1002/hep.27735.
95. Singal AG, Mukherjee A, Elmunzer BJ, Higgins PD, Lok AS, Zhu J, et al. Machine learning algorithms outperform conventional regression models in predicting development of hepatocellular carcinoma. Am J Gastroenterol. 2013;108(11):1723–30. https://doi.org/10.1038/ajg.2013.332.
96. Cho JY, Paik YH, Park HC, Yu JI, Sohn W, Gwak GY, et al. The feasibility of combined transcatheter arterial chemoembolization and radiotherapy for advanced hepatocellular carcinoma. Liver Int. 2014;34(5):795–801. https://doi.org/10.1111/liv.12445.

97. Ganne-Carrie N, Layese R, Bourcier V, Cagnot C, Marcellin P, Guyader D, et al. Nomogram for individualized prediction of hepatocellular carcinoma occurrence in hepatitis C virus cirrhosis (ANRS CO12 CirVir). Hepatology. 2016;64(4):1136–47. https://doi.org/10.1002/hep.28702.
98. Nakagomi R, Tateishi R, Masuzaki R, Soroida Y, Iwai T, Kondo M, et al. Liver stiffness measurements in chronic hepatitis C: Treatment evaluation and risk assessment. J Gastroenterol Hepatol. 2018. https://doi.org/10.1111/jgh.14530.
99. Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009;136(1):138–48. https://doi.org/10.1053/j.gastro.2008.09.014.
100. El-Serag HB, Kanwal F, Davila JA, Kramer J, Richardson P. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. Gastroenterology. 2014;146(5):1249–55 e1. https://doi.org/10.1053/j.gastro.2014.01.045.
101. Chang KC, Wu YY, Hung CH, Lu SN, Lee CM, Chiu KW, et al. Clinical-guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. Br J Cancer. 2013;109(9):2481–8. https://doi.org/10.1038/bjc.2013.564.
102. Ikeda M, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, et al. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. J Gastroenterol. 2005;40(2):148–56. https://doi.org/10.1007/s00535-004-1519-2.
103. Chang KC, Hung CH, Lu SN, Wang JH, Lee CM, Chen CH, et al. A novel predictive score for hepatocellular carcinoma development in patients with chronic hepatitis C after sustained response to pegylated interferon and ribavirin combination therapy. J Antimicrob Chemother. 2012;67(11):2766–72. https://doi.org/10.1093/jac/dks269.
104. Casadei Gardini A, Foschi FG, Conti F, Petracci E, Vukotic R, Marisi G, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. Dig Liver Dis. 2018. https://doi.org/10.1016/j.dld.2018.09.016.
105. Flemming JA, Yang JD, Vittinghoff E, Kim WR, Terrault NA. Risk prediction of hepatocellular carcinoma in patients with cirrhosis: the ADRESS-HCC risk model. Cancer. 2014;120(22):3485–93. https://doi.org/10.1002/cncr.28832.
106. Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology. 2003;37(3):520–7. https://doi.org/10.1053/jhep.2003.50093.
107. Aoki T, Iijima H, Tada T, Kumada T, Nishimura T, Nakano C, et al. Prediction of development of hepatocellular carcinoma using a new scoring system involving virtual touch quantification in patients with chronic liver diseases. J Gastroenterol. 2017;52(1):104–12. https://doi.org/10.1007/s00535-016-1228-7.
108. Wen CP, Lin J, Yang YC, Tsai MK, Tsao CK, Etzel C, et al. Hepatocellular carcinoma risk prediction model for the general population: the predictive power of transaminases. J Natl Cancer Inst. 2012;104(20):1599–611. https://doi.org/10.1093/jnci/djs372.
109. Konerman MA, Zhang Y, Zhu J, Higgins PD, Lok AS, Waljee AK. Improvement of predictive models of risk of disease progression in chronic hepatitis C by incorporating longitudinal data. Hepatology. 2015;61(6):1832–41. https://doi.org/10.1002/hep.27750.
110. Lens S, Torres F, Puigvehi M, Marino Z, Londono MC, Martinez SM, et al. Predicting the development of liver cirrhosis by simple modelling in patients with chronic hepatitis C. Aliment Pharmacol Ther. 2016;43(3):364–74. https://doi.org/10.1111/apt.13472.
111. Vilar-Gomez E, Calzadilla-Bertot L, Friedman SL, Gra-Oramas B, Gonzalez-Fabian L, Lazo-Del Vallin S, et al. Serum biomarkers can predict a change in liver fibrosis 1 year after lifestyle intervention for biopsy-proven NASH. Liver Int. 2017;37(12):1887–96. https://doi.org/10.1111/liv.13480.
112. Irvine KM, Wockner LF, Shanker M, Fagan KJ, Horsfall LU, Fletcher LM, et al. The Enhanced liver fibrosis score is associated with clinical outcomes and disease progression in patients with chronic liver disease. Liver Int. 2016;36(3):370–7. https://doi.org/10.1111/liv.12896.

113. Chen K, Shi W, Xin Z, Wang H, Zhu X, Wu X, et al. Replication of genome wide association studies on hepatocellular carcinoma susceptibility loci in a Chinese population. PLoS One. 2013;8(10):e77315. https://doi.org/10.1371/journal.pone.0077315.
114. Kozuka R, Enomoto M, Sato-Matsubara M, Yoshida K, Motoyama H, Hagihara A, et al. Association between HLA-DQA1/DRB1 polymorphism and development of hepatocellular carcinoma during entecavir treatment. J Gastroenterol Hepatol. 2018. https://doi.org/10.1111/jgh.14454.
115. Tanabe KK, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA. 2008;299(1):53–60. https://doi.org/10.1001/jama.2007.65.
116. Abu Dayyeh BK, Yang M, Fuchs BC, Karl DL, Yamada S, Sninsky JJ, et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology. 2011;141(1):141–9. https://doi.org/10.1053/j.gastro.2011.03.045.
117. Jiang G, Yu K, Shao L, Yu X, Hu C, Qian P, et al. Association between epidermal growth factor gene +61A/G polymorphism and the risk of hepatocellular carcinoma: a meta-analysis based on 16 studies. BMC Cancer. 2015;15:314. https://doi.org/10.1186/s12885-015-1318-6.
118. Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, et al. Genetic variation near interleukin 28B and the risk of hepatocellular carcinoma in patients with chronic hepatitis C. J Gastroenterol. 2014;49(7):1152–62. https://doi.org/10.1007/s00535-013-0858-2.
119. Chang KC, Tseng PL, Wu YY, Hung HC, Huang CM, Lu SN, et al. A polymorphism in interferon L3 is an independent risk factor for development of hepatocellular carcinoma after treatment of hepatitis C virus infection. Clin Gastroenterol Hepatol. 2015;13(5):1017–24. https://doi.org/10.1016/j.cgh.2014.10.035.
120. Hodo Y, Honda M, Tanaka A, Nomura Y, Arai K, Yamashita T, et al. Association of interleukin-28B genotype and hepatocellular carcinoma recurrence in patients with chronic hepatitis C. Clin Cancer Res. 2013;19(7):1827–37. https://doi.org/10.1158/1078-0432.CCR-12-1641.
121. Tong HV, Toan NL, Song LH, Bock CT, Kremsner PG, Velavan TP. Hepatitis B virus-induced hepatocellular carcinoma: functional roles of MICA variants. J Viral Hepat. 2013;20(10):687–98. https://doi.org/10.1111/jvh.12089.
122. Huang CF, Wang SC, Yeh ML, Huang CI, Tsai PC, Lin ZY, et al. Association of serial serum major histocompatibility complex class I chain-related A measurements with hepatocellular carcinoma in chronic hepatitis C patients after viral eradication. J Gastroenterol Hepatol. 2019;34(1):249–55. https://doi.org/10.1111/jgh.14359.
123. Guyot E, Sutton A, Rufat P, Laguillier C, Mansouri A, Moreau R, et al. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. J Hepatol. 2013;58(2):312–8. https://doi.org/10.1016/j.jhep.2012.09.036.
124. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol. 2014;61(1):75–81. https://doi.org/10.1016/j.jhep.2014.02.030.
125. Falleti E, Fabris C, Cmet S, Cussigh A, Bitetto D, Fontanini E, et al. PNPLA3 rs738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int. 2011;31(8):1137–43. https://doi.org/10.1111/j.1478-3231.2011.02534.x.
126. Yang J, Trepo E, Nahon P, Cao Q, Moreno C, Letouze E, et al. PNPLA3 and TM6SF2 variants as risk factors of hepatocellular carcinoma across various etiologies and severity of underlying liver diseases. Int J Cancer. 2019;144(3):533–44. https://doi.org/10.1002/ijc.31910.
127. Stickel F, Buch S, Nischalke HD, Weiss KH, Gotthardt D, Fischer J, et al. Genetic variants in PNPLA3 and TM6SF2 predispose to the development of hepatocellular carcinoma in individuals with alcohol-related cirrhosis. Am J Gastroenterol. 2018;113(10):1475–83. https://doi.org/10.1038/s41395-018-0041-8.

128. Falleti E, Cussigh A, Cmet S, Fabris C, Toniutto P. PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis. Dig Liver Dis. 2016;48(1):69–75. https://doi.org/10.1016/j.dld.2015.09.009.
129. Nahon P, Sutton A, Rufat P, Ziol M, Thabut G, Schischmanoff PO, et al. Liver iron, HFE gene mutations, and hepatocellular carcinoma occurrence in patients with cirrhosis. Gastroenterology. 2008;134(1):102–10. https://doi.org/10.1053/j.gastro.2007.10.038.
130. Hoshida Y, Villanueva A, Sangiovanni A, Sole M, Hur C, Andersson KL, et al. Prognostic gene expression signature for patients with hepatitis C-related early-stage cirrhosis. Gastroenterology. 2013;144(5):1024–30. https://doi.org/10.1053/j.gastro.2013.01.021.
131. King LY, Canasto-Chibuque C, Johnson KB, Yip S, Chen X, Kojima K, et al. A genomic and clinical prognostic index for hepatitis C-related early-stage cirrhosis that predicts clinical deterioration. Gut. 2015;64(8):1296–302. https://doi.org/10.1136/gutjnl-2014-307862.
132. Kim JH, Sohn BH, Lee HS, Kim SB, Yoo JE, Park YY, et al. Genomic predictors for recurrence patterns of hepatocellular carcinoma: model derivation and validation. PLoS Med. 2014;11(12):e1001770. https://doi.org/10.1371/journal.pmed.1001770.
133. Finkin S, Yuan D, Stein I, Taniguchi K, Weber A, Unger K, et al. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. Nat Immunol. 2015;16(12):1235–44. https://doi.org/10.1038/ni.3290.
134. Ji J, Eggert T, Budhu A, Forgues M, Takai A, Dang H, et al. Hepatic stellate cell and monocyte interaction contributes to poor prognosis in hepatocellular carcinoma. Hepatology. 2015;62(2):481–95. https://doi.org/10.1002/hep.27822.
135. Zhang DY, Goossens N, Guo J, Tsai MC, Chou HI, Altunkaynak C, et al. A hepatic stellate cell gene expression signature associated with outcomes in hepatitis C cirrhosis and hepatocellular carcinoma after curative resection. Gut. 2016;65(10):1754–64. https://doi.org/10.1136/gutjnl-2015-309655.
136. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. Gastroenterology. 2018;154(6):1706–18 e1. https://doi.org/10.1053/j.gastro.2018.01.064.
137. Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. Hepatol Int. 2008;2(1):17–30. https://doi.org/10.1007/s12072-007-9038-x.
138. Xing H, Zheng YJ, Han J, Zhang H, Li ZL, Lau WY, et al. Protein induced by vitamin K absence or antagonist-II versus alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: a systematic review with meta-analysis. Hepatobiliary Pancreat Dis Int. 2018;17(6):487–95. https://doi.org/10.1016/j.hbpd.2018.09.009.
139. Yang JD, Addissie BD, Mara KC, Harmsen WS, Dai J, Zhang N, et al. GALAD score for hepatocellular carcinoma detection in comparison to liver ultrasound and proposal of GALADUS score. Cancer Epidemiol Biomark Prev. 2018. https://doi.org/10.1158/1055-9965.EPI-18-0281.
140. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016;14(6):875–86 e6. https://doi.org/10.1016/j.cgh.2015.12.042.
141. Mehta AS, Lau DT, Wang M, Islam A, Nasir B, Javaid A, et al. Application of the Doylestown algorithm for the early detection of hepatocellular carcinoma. PLoS One. 2018;13(8):e0203149. https://doi.org/10.1371/journal.pone.0203149.
142. Jia X, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. Arch Med Res. 2014;45(7):580–8. https://doi.org/10.1016/j.arcmed.2014.11.002.
143. Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol. 2011;29(36):4781–8. https://doi.org/10.1200/JCO.2011.38.2697.

144. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol. 2012;13(8):817–26. https://doi.org/10.1016/S1470-2045(12)70233-4.
145. Zhu WW, Guo JJ, Guo L, Jia HL, Zhu M, Zhang JB, et al. Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. Clin Cancer Res. 2013;19(14):3944–54. https://doi.org/10.1158/1078-0432.CCR-12-3363.
146. Vongsuvanh R, van der Poorten D, Iseli T, Strasser SI, McCaughan GW, George J. Midkine increases diagnostic yield in AFP negative and NASH-related hepatocellular carcinoma. PLoS One. 2016;11(5):e0155800. https://doi.org/10.1371/journal.pone.0155800.
147. Sun Y, Gao G, Cai J, Wang Y, Qu X, He L, et al. Annexin A2 is a discriminative serological candidate in early hepatocellular carcinoma. Carcinogenesis. 2013;34(3):595–604. https://doi.org/10.1093/carcin/bgs372.
148. Liu XE, Desmyter L, Gao CF, Laroy W, Dewaele S, Vanhooren V, et al. N-glycomic changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. Hepatology. 2007;46(5):1426–35. https://doi.org/10.1002/hep.21855.
149. Sun T, Tang Y, Sun D, Bu Q, Li P. Osteopontin versus alpha-fetoprotein as a diagnostic marker for hepatocellular carcinoma: a meta-analysis. Onco Targets Ther. 2018;11:8925–35. https://doi.org/10.2147/OTT.S186230.
150. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol. 2005;43(6):1007–12. https://doi.org/10.1016/j.jhep.2005.05.028.

Chapter 2
Hepatocellular Carcinoma Surveillance and Staging

Amit G. Singal, Neehar D. Parikh, Nicole E. Rich, Binu V. John, and Anjana Pillai

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide [1]. Despite improvement over time, the majority of HCC patients in the United States continue to be diagnosed at advanced stages, and prognosis remains poor, with median survival of less than 6 months [2]. One of the strongest predictors of overall survival is early tumor detection. Patients diagnosed with early-stage HCC are eligible for curative treatments including liver transplantation, surgical resection, or local ablative therapy and can achieve 5-year survival exceeding 70%. In contrast, patients with advanced HCC are only amenable to palliative therapies and have a particularly poor prognosis, with median survival typically below 1 year. Thus, early detection and accurate staging to guide treatment decisions are essential to prolong survival of patients with HCC.

A. G. Singal (✉) · N. E. Rich
Division of Digestive and Liver Diseases, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA
e-mail: amit.singal@utsouthwestern.edu

N. D. Parikh
Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

B. V. John
Department of Medicine, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA, USA

A. Pillai
Department of Internal Medicine, University of Chicago, Chicago, IL, USA

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_2

HCC Surveillance

Since most patients with HCC are asymptomatic when tumors are at an early stage, routine surveillance (or screening) in patients at high risk for the development of HCC is particularly important. The goal of HCC surveillance among at-risk patients is to detect HCC at an early stage when treatment options would be most effective in resulting in prolonged patient survival. Several organizations have issued evidence-based recommendations for HCC surveillance in populations at high risk of the disease (Table 2.1) [3–5].

The American Association for the Study of Liver Diseases (AASLD) guidelines, updated in 2017, recommend HCC surveillance every 6 months using abdominal ultrasound with or without the serum biomarker, alpha-fetoprotein (AFP) in at-risk individuals, including subgroups of patients with hepatitis B virus (HBV) and all patients with cirrhosis from any etiology [5]. Surveillance is not recommended in patients with Child-Pugh C cirrhosis unless they are awaiting liver transplantation, given the low probability of treatment eligibility. Recommendations from the European Association for the Study of the Liver (EASL), Asian Pacific Association for the Study of the Liver (APASL), and National Comprehensive Cancer Network (NCCN) also recommend surveillance every 6 months [3, 4]. However, there are key differences between professional society guidelines (Table 2.2); for example, EASL includes patients with chronic HCV with F3 fibrosis among at-risk patients requiring surveillance and recommends surveillance using ultrasound alone without AFP. Though HCC has been reported in some patients with noncirrhotic NASH, none of the guidelines recommend surveillance in patients with NASH in the absence of cirrhosis.

Table 2.1 Populations in whom hepatocellular carcinoma surveillance is recommended

Surveillance recommended
Asian male hepatitis B carriers over age 40
Asian female hepatitis B carriers over age 50
African blacks with hepatitis B
Hepatitis B carriers with family history of HCC
Cirrhosis related to hepatitis B
Cirrhosis related to hepatitis C
Cirrhosis related to genetic hemochromatosis
Cirrhosis related to other etiologies
Surveillance benefits uncertain
Hepatitis B carriers younger than 40 (males) or 50 (females)
Hepatitis B carriers who contacted infection via horizontal transmission
Chronic hepatitis C infection without cirrhosis
Nonalcoholic fatty liver patients without cirrhosis

Table 2.2 Professional society hepatocellular carcinoma surveillance recommendations

Professional society guideline	At-risk patient population	Frequency of surveillance	Recommended surveillance tests
American Association for the Study of Liver Diseases (AASLD)	Patients with cirrhosis except Child-Pugh C unless awaiting liver transplantation; noncirrhotic HBV carriers: Asian females >50 years, Asian males >40 years, Africans/ North American blacks, and family history of HCC	Every 6 months	Ultrasonography with or without AFP testing
National Comprehensive Cancer Network® (NCCN®)	Patients with cirrhosis; noncirrhotic HBV carriers	Every 6 months	Ultrasonography with or without AFP testing
US Department of Veterans Affairs	Patients with cirrhosis; noncirrhotic HBV carriers: Asian females >50 years, Asian males >40 years, Africans >20 years, and family history of HCC	Every 6–12 months	Ultrasonography and serum AFP measurement
European Association for the Study of the Liver (EASL)	Patients with cirrhosis, Child-Pugh A and B, or Child-Pugh C awaiting transplantation; noncirrhotic HBV carriers with active hepatitis or family history of HCC; and noncirrhotic patients with chronic hepatitis C and fibrosis stage F3	Every 6 months	Ultrasonography alone
Asian Pacific Association for the Study of the Liver (APASL)	Patients with cirrhosis; noncirrhotic HBV carriers: Asian females >50 years, Asian males >40 years, Africans >20 years, and family history of HCC	Every 6 months	Ultrasonography and serum AFP measurement

Benefits of HCC Surveillance

Professional society recommendations for HCC surveillance are supported by several studies that have demonstrated improved early tumor detection, curative treatment receipt, and overall survival. Studies on surveillance should be evaluated separately for patients with chronic HBV versus those with cirrhosis given differences in patient populations, HCC risk, surveillance test effectiveness, and curative treatment eligibility.

Patients with Chronic HBV Infection

There have been two large randomized controlled trials comparing surveillance every 6 months to no surveillance in chronic HBV patients [6, 7]. In both studies, patients randomized to surveillance were significantly more likely to be detected at

an early stage, were more likely to undergo curative therapy, and had better overall survival. The first study included 17,820 HBV-infected persons who were randomized to surveillance or no surveillance and followed for an average of 14.4 months [6]. Of patients randomized to surveillance who developed HCC, 29 (76.3%) were detected at an early stage, whereas none of the 18 patients who developed HCC in the no-surveillance group were detected at an early stage ($p < 0.01$). Similarly, a higher proportion of patients in the surveillance group underwent curative therapy, with 24 patients having resection in the surveillance group compared to none in the no-surveillance group ($p < 0.05$). Accordingly, the 1- and 2-year survival rates for HCC patients in the surveillance group were 88.1% and 77.5%, respectively, compared to 0% at 1 year for HCC patients in the no-surveillance group ($p < 0.01$). Although this study was limited by lead-time bias, this would only account for a survival difference of 5.4 months, so data from this study still suggests surveillance reduces HCC-related mortality. The second trial included 18,816 HBV carriers who were randomized to surveillance or no surveillance [7]. HCC was detected at an early stage in 45% of the 86 surveillance-group patients who developed HCC, compared to none of the 67 patients who developed HCC in the no-surveillance group ($p < 0.01$). HCC-related mortality of patients undergoing surveillance was significantly lower than that of the no-surveillance (83.2 vs. 131.5 per 100,000, $p < 0.01$), with a hazard ratio of 0.63 (95% CI 0.41–0.98). Taken together, these randomized controlled trials provide level I evidence that HCC surveillance among patients with chronic HBV infection improves early tumor detection and reduces cancer-related mortality.

Patients with Cirrhosis

Given the lower sensitivity of abdominal ultrasound for detection of HCC in a nodular cirrhotic liver, added comorbidities in this patient population, and fewer curative treatment options for cirrhosis patients, one cannot extrapolate surveillance data from patients with chronic HBV infection who are predominantly noncirrhotic to patients with cirrhosis. Although there have not been randomized controlled trials in patients with cirrhosis, several cohort studies have demonstrated an association between HCC surveillance and increased early detection, curative treatment receipt, and improved overall survival [8]. Although these cohort studies have limitations including potential for confounders, length-time bias, and lead-time bias (Fig. 2.1) [9], they provide consistent evidence of likely benefit from HCC surveillance in cirrhosis patients.

In a retrospective analysis of a prospective cohort among 451 HCC patients, patients who underwent surveillance had significantly prolonged survival compared to those who did not undergo surveillance, after adjusting for lead-time bias [10]. The greatest benefit was seen in patients with Child-Pugh A cirrhosis although patients with Child-Pugh B cirrhosis still derived a survival benefit. There was not an observed survival benefit of HCC surveillance in patients with Child-Pugh C cirrhosis given the high competing risk of dying from cirrhosis-related complications.

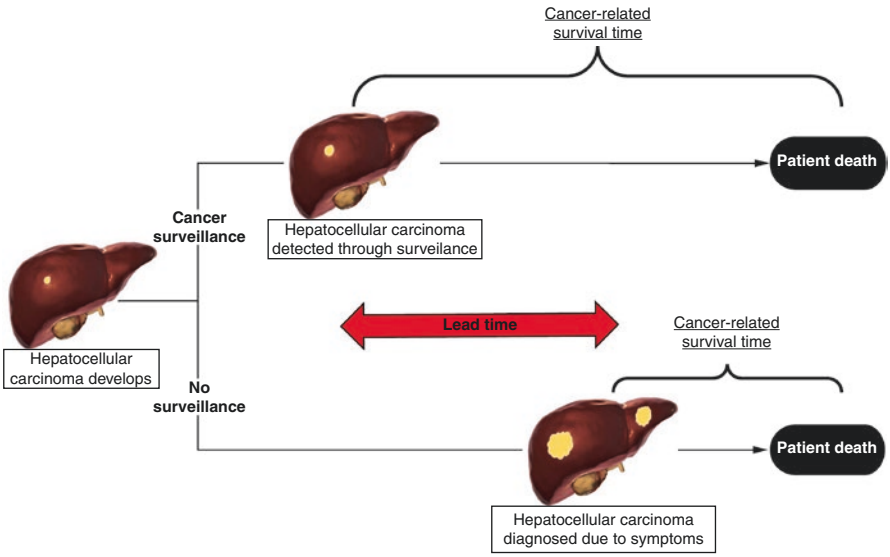

Fig. 2.1 Lead-time bias for cohort study showing association between HCC surveillance and survival benefit

Similarly, a retrospective analysis among 1480 HCC patients from the Department of Veterans Affairs (VA) database demonstrated HCC surveillance at 0–6 months and 7–24 months prior to HCC diagnosis was associated with significantly lower mortality risk (HR 0.71, 95% CI 0.62–0.82) compared to no surveillance [11].

A systematic review and meta-analysis of 47 studies (including a total of 15,158 patients with cirrhosis) demonstrated surveillance is associated with increased early tumor detection and curative treatment receipt as well as improved overall survival [8]. Patients who had undergone surveillance were significantly more likely to have HCC diagnosed at an early stage (OR 2.08, 95% CI 1.80–2.37) and undergo curative treatment receipt (OR 2.24, 95% CI 1.99–2.52). The pooled proportion of patients with early-stage HCC was 70.9% among patients undergoing surveillance, compared with only 29.9% of those without surveillance. Similarly, a significantly higher proportion of patients undergoing surveillance underwent curative treatment receipt compared to those who did not receive prior surveillance (51.6% vs. 23.7%). Surveillance was also significantly associated with improved overall survival (OR 1.90, 95% CI 1.67–2.17), with a pooled 3-year survival of 50.8% among those who underwent surveillance versus 27.9% for those who presented symptomatically or were diagnosed incidentally. Among the subset of studies that adjusted for lead-time bias, the association between HCC surveillance and improved survival was sustained (3-year survival 39.7% vs. 29.1%). Another systematic review of 2 trials and 18 observational studies similarly concluded HCC surveillance is associated with early tumor detection; however, the authors were unable to conclude if there was a survival benefit due to study limitations including unmeasured confounders and length-time bias [9].

Evidence in support of HCC surveillance in patients with cirrhosis continues to accumulate, with several recent studies also demonstrating HCC surveillance benefits.

A recent analysis from the Netherlands evaluated the effectiveness of HCC surveillance in five Dutch academic centers and showed that in those patients undergoing HCC surveillance, tumors were detected at a smaller size (2.7 cm vs. 6.0 cm) and earlier stage (61% vs. 21%), more therapeutic options were offered, and 1-, 3-, and 5-year survival rates were significantly higher after adjusting for lead-time bias [12]. Similarly, an analysis among 374 patients followed at four US centers found HCC surveillance was associated with significantly improved early detection (63.1% vs. 36.4%) and curative treatment receipt (31% vs.13%). HCC surveillance was associated with improved survival (HR 0.59, 95% CI 0.37–0.93) after adjusting for demographics, Child-Pugh class, and performance status and accounting for lead-time bias [13]. Finally, an analysis of HCC surveillance patterns in patients with HCV- or HBV-associated cirrhosis conducted among 35 centers in France showed that semi-annual surveillance was associated with early diagnosis and more curative treatment options. In addition, after adjusting for lead-time bias, overall survival was longer in the surveillance group (53.2 vs. 25.4 months $p = 0.01$) compared to the group that did not adhere to surveillance guidelines [14].

Harms of HCC Surveillance

The value of a cancer screening program must weigh benefits against potential screening-related harms. Multiple types of harms should be considered when considering a cancer screening program including the potential for physical, financial, and psychological harms [15, 16]. Physical harms can result from screening or follow-up testing and extend beyond medical complications to include discomfort. Financial harms can include anticipated or real costs of screening and diagnostic evaluation plus indirect costs such as missed work. There are also opportunity costs, including patient distraction from other health-related activities or self-care and misallocation of limited resources, such as radiology facilities, from a system perspective. Psychological harms can occur at any step of the screening process and include anticipation or fear of abnormal results, reactions of depression, anxiety, or cancer-specific worry after positive results, and psychological effects of being labeled with a diagnosis. Although HCC surveillance using ultrasound and AFP has minimal discomfort and no direct physical harms, there are potential "downstream" harms associated with diagnostic evaluation protocols.

To date, there have been few studies quantifying HCC surveillance harms in patients with cirrhosis. In a single-center retrospective cohort study among 680 cirrhosis patients undergoing HCC screening over a 3-year period, screening-related physical harms were reported in 187 (27.5%) patients, with 66 (9.7%) having multiple CT/MRI exams [17]. Three (0.4%) patients underwent angiogram or biopsy after multiple (≥7) CT/MRI exams, with 1 hospitalized for postbiopsy bleeding.

Harms increased from 11.9% among those with 1 screening exam to 29.6% among those with ≥2 screening exams. The most common trigger was false-positive screening tests (ultrasound 34% and AFP 27%), but 39% had harms due to indeterminate results. Screening harms were associated with elevated alanine transaminase (ALT), portal hypertension, and receipt of subspecialty gastroenterology care. In another single-center study among 999 patients undergoing HCC surveillance over a median follow-up of 2.2 years, 256 patients were found to have abnormal imaging – 69 who were diagnosed with HCC and 187 with an indeterminate nodule (i.e., lesion greater than 1 cm in diameter that could not be categorized as definitely benign or definite HCC on cross-sectional imaging). Among those with indeterminate nodules, 18 (9.6%) did not undergo further diagnostic evaluation, 132 (70.6%) returned to ultrasound surveillance after negative CT/MR imaging (median 2; IQR 1–3), and 37 (19.8%) continued CT/MR-based imaging (median 2; IQR 1–2). Of those who underwent diagnostic evaluation with CT/MRI, nearly half (47.9%) had multiple CT/MRI exams and 11 underwent invasive evaluation including biopsy. Future studies evaluating HCC surveillance should consider both benefits and harms to better characterize its overall value.

Surveillance Tests

An ideal surveillance test would be highly sensitive and specific for early HCC and undetectable in premalignant liver disease. Additionally, tests that are easily measurable, reproducible, and minimally invasive are better accepted by both patients and physicians. Currently, both radiographic and serologic tests are used for HCC surveillance.

Radiographic Surveillance Tests

Liver ultrasound has been long regarded as a standard surveillance test for HCC and is one of the most commonly used. Its advantages include being noninvasive and relatively inexpensive, and it poses no risk of contrast or radiation exposure. In one of the randomized trials evaluating surveillance among HBV patients, the sensitivity of ultrasound was 84% for HCC at any stage and 63% for early-stage HCC [18]. However, the performance of ultrasound appears to be worse in patients with cirrhosis. In the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial, a large multicenter study in the United States, the sensitivity of ultrasound for early-stage tumors was substantially lower. Of 39 patients with HCC analyzed in a nested case-control study, only 14 (35.9%) tumors were detected at an early stage by ultrasound [19]. These results were also confirmed in a large prospective single-center cohort study, in which the sensitivity of ultrasound for early-stage HCC was only 31.7% [20]. A meta-analysis of studies performed among cirrhotic

patients reported a pooled sensitivity of 84% (95% CI 76–92%) and a pooled specificity of 91% (95% CI 86–94%) for detection of HCC at any stage. In a subgroup analysis of studies that examined early-stage HCC detection, the pooled sensitivity of ultrasound was only 47% (95% CI 33–61%) for detection of early-stage HCC [21]. There was a wide range in sensitivities for any stage HCC detection (28–100%) as well as early HCC detection (21–89%).

The wide range in sensitivities for HCC detection may reflect the operator-dependent nature of ultrasound and heterogeneity in the patient populations included in the studies. Detection of HCC in the background of a nodular cirrhotic liver is particularly challenging due to the presence of fibrous septa and regenerative nodules, which appear as a coarse pattern on ultrasound and may mask the presence of a small tumor. The operator dependency of ultrasound and the importance of high-quality equipment for good performance have been emphasized in consensus guidelines. To maximize ultrasound efficacy, special training for those performing ultrasounds for HCC surveillance has been advocated.

The performance of ultrasound can also be impacted by several patient-level factors, with particularly suboptimal performance in obese patients and those with more advanced cirrhosis. In a retrospective analysis of 941 cirrhotic patients undergoing HCC surveillance, over 20% of ultrasound exams were determined to be of inadequate quality for surveillance [22]. Factors correlated with ultrasound inadequacy included male sex, obesity and morbid obesity, alcohol or NASH etiology of cirrhosis, advanced liver disease (Child-Pugh class B cirrhosis), inpatient status, and elevated alanine transaminase (ALT). Rib shadowing and poor beam penetration were the most common causes for unsatisfactory liver visualization and diminished ultrasound quality. Likewise, another study found male sex, Child-Pugh class B cirrhosis, and elevated AFP levels were significantly correlated with surveillance failure in patients with cirrhosis, which was defined as a tumor detected beyond an early stage or missed on ultrasound and later identified by CT or MRI [23]. In a retrospective analysis of the HALT-C data, the most common reason for not diagnosing HCC at an early stage was an "absence of detection," i.e., ultrasound failing to detect HCC lesions [24]. Taken together, these data clearly highlight the need for better HCC surveillance tools to improve detection of early-stage tumors.

Cross-sectional imaging modalities, such as CT or MRI, would be anticipated to have high accuracy based on data for HCC diagnosis. However, there have been few data evaluating CT or MRI for surveillance purposes, with only two studies evaluating CT-based surveillance and two evaluating MRI-based surveillance. In a single-center randomized controlled trial comparing ultrasound- and CT-based surveillance in patients with cirrhosis, the sensitivity and specificity of CT for any stage detection were 87.5% (95% CI 50.8–99.9%) and 87.5% (95% CI 77.7–93.5%), respectively; however, the sensitivity of CT for early HCC detection was only 62.5% (95% CI 30.4–86.5%) and did not significantly differ from that of ultrasound [25]. The other study by Van Thiel and colleagues reported sensitivity and specificity of CT for any stage detection but did not report performance characteristics for early HCC detection [26]. Further, CT-based surveillance is also likely limited by potential harms including radiation exposure and contrast-induced nephrotoxicity. The two

studies evaluating MRI had a pooled sensitivity and specificity for any HCC detection of 83.1% (95% CI 72.0–90.5%) and 89.1% (95% CI 86.5–91.3%), respectively [27, 28]. In the PRIUS study, a prospective cohort study of 407 cirrhotic patients comparing MRI- and ultrasound-based surveillance, MRI-based surveillance resulted in significantly higher early-stage HCC detection (83.7% vs. 25.6%; $p < 0.001$) compared with ultrasound [27]. In addition, MRI had significantly fewer false-positive findings (3.0% vs. 5.6%; $p = 0.004$). Although these data suggest MRI-based surveillance could improve early tumor detection, this is likely not cost-effective if implemented in all at-risk patients and might best be reserved for the subset of patients who are prone to ultrasound failure.

Serologic Surveillance Tests

The addition of biomarkers to ultrasound for surveillance has been proposed as a means of increasing the sensitivity of surveillance, particularly with regard to early tumor detection. The best studied biomarker to date is AFP, a glycoprotein that is expressed by fetal hepatocytes and/or poorly differentiated HCC cells.

Assessment of AFP has several advantages including being easy to perform, inexpensive, and broadly available. However, the optimal threshold for AFP positivity is under debate. A level of 20 ng/mL has become the most commonly used cutoff to trigger further evaluation in clinical practice, although this value was derived from a study in which only one-third of patients had early-stage HCC. For the detection of early-stage HCC, the sensitivity of AFP drops considerably. At a cutoff value of 20 ng/mL, the sensitivity of AFP for any stage HCC is approximately 60%, but sensitivity for early-stage tumors is only 32–49% [29]. Further, false-negative and false-positive findings are not uncommon, each occurring at a rate of about 20–40%. AFP may be elevated in the setting of chronic liver disease, particularly in patients with significant elevations of transaminases, and in some patients with non-HCC malignancies such as cholangiocarcinoma. A systematic review of five studies evaluating AFP at this level in cirrhotic patients showed sensitivities ranging from 41% to 65% and specificities ranging from 80% to 94% for HCC at any stage [30]. A multicenter phase 2 case-control biomarker study among 417 patients with cirrhosis but no HCC and 419 patients with HCC (49.6% early-stage tumors) showed that AFP, using a lower cutoff of 10.9 ng/mL, had improved sensitivity of 66% for early-stage HCC compared to the traditional cutoff of 20 ng/mL [31].

Current evidence suggests that ultrasound in combination with AFP is likely the most effective strategy for HCC surveillance in patients with cirrhosis. A prospective cohort study among 446 patients with cirrhosis found the sensitivity of ultrasound alone for early HCC detection was 31.7%, compared to 63.4% when using the two tests in combination [20]. A secondary analysis of a multicenter randomized controlled trial in France comparing 3- and 6-month surveillance intervals also found a benefit of using AFP in combination with ultrasound (sensitivity 74.8% vs. 65.0%), although the difference was not statistically significant (RR 1.15, 95% CI 0.97–1.35)

[32]. Similarly, a secondary analysis of the PRIUS study from Korea found a nonsignificant improvement in sensitivity using ultrasound with AFP than ultrasound alone (RR 1.27, 95% CI 0.65–2.50) although sensitivities were very low in both groups (32.6% vs. 25.6%, respectively) [27]. A meta-analysis of studies evaluating this debate found ultrasound with AFP had significantly higher sensitivity than ultrasound alone (RR 1.23, 95% CI 1.08–1.41) [21]. The pooled sensitivities of ultrasound with and without AFP for early-stage HCC were 63% (95% CI 48–75%) and 45% (95% CI 30–62%), respectively ($p = 0.002$). The benefit of AFP as an adjunct test to ultrasound was consistent across subgroups including prospective studies (RR 1.28, 95% CI 1.09–1.52), studies conducted in the United States (RR 1.70, 95% CI 1.18–2.44), and studies conducted after the year 2000 (RR 1.27, 95% CI 1.05–1.52).

There have been several methods proposed to further improve AFP accuracy for HCC detection. AFP levels can fluctuate with exacerbations of underlying liver disease so often have lower specificity in those with active viral hepatitis and higher specificity in patients with nonviral causes of liver disease and after successful antiviral treatment due to decreased hepatitis activity [33]. In a retrospective cohort study of 1128 patients with cirrhosis, AFP was significantly more accurate in HCV-negative patients than those with active HCV infection (c-statistic 0.89 vs. 0.83, $p = 0.007$) [34]. Therefore, Gopal and colleagues suggested tailoring AFP cutoffs by liver disease etiology may maximize accuracy including cutoffs of 59 ng/mL for HCV-positive patients and 11 ng/mL for HCV-negative patients. Another proposed method to improve AFP accuracy is incorporating other patient factors to develop AFP-adjusted algorithms. In another study among 11,721 patient with HCV-related cirrhosis, an AFP-adjusted algorithm incorporating platelet count, ALT level, and patient age was developed to improve predictive value of AFP for identifying patients likely to develop HCC within 6 months [35]. Similarly, the Doylestown algorithm is an AFP-adjusted algorithm that includes age, sex, alkaline phosphatase, and ALT [36]. External validation of the Doylestown algorithm in a cohort of >2700 patients with cirrhosis demonstrated increased performance (AUC) for HCC detection by 4–20% compared with AFP alone. Finally, longitudinal AFP measurements have been evaluated to discriminate benign changes in AFP from changes that reflect true development of HCC and improve accuracy compared to single-threshold measurements. In a case-control study that employed 193 patients with HCC and 74 patients with cirrhosis, longitudinal changes in AFP was shown to be more sensitive for HCC detection than a single-threshold method. For an average progressive monthly increase of ≥7 ng/mL in AFP levels, sensitivity and specificity were 71.4% and 100%, respectively. In a secondary analysis of the HALT-C Trial, incorporation of the standard deviation and rise of AFP significantly improved accuracy for detecting HCC compared to only using the most recent AFP level (c-statistic 0.81 vs. 0.76; $p < 0.001$) [37]. In another analysis of the HALT-C Trial cohort, a parametric empirical Bayes screening algorithm was similarly shown to be more effective at detecting HCC in patients with cirrhosis (77.1% from 60.4%, $p < 0.005$) than the single-threshold method [38].

Other promising biomarkers are in development for the diagnosis of HCC, but most have only been evaluated in phase II biomarker studies, so their utility as HCC surveillance markers is not yet known. Des-gamma-carboxy prothrombin (DCP), an

abnormal prothrombin protein that is generated as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant hepatic cells, and lens culinaris agglutinin-reactive AFP (AFP-L3), an isoform of AFP, have both been approved by the FDA for predicting risk but not for HCC surveillance. The relative risk of HCC development is increased 7-fold with AFP-L3 ratios ≥10% and 4.8-fold with elevated DCP ≥7.5 ng/mL. Several prospective cohort studies in patients with cirrhosis have evaluated DCP for detecting HCC and found sensitivities ranging from 23% to 57% compared to 14–71% for AFP [39–41]. In a nested case-control study among patients in the HALT-C Trial, DCP and AFP had sensitivities of 74% and 61%, respectively, for HCC at any stage, which was increased to 91% by using the two markers in combination [19]. Prospective studies evaluating AFP-L3 in patients with cirrhosis have demonstrated sensitivities ranging from 35% to 75% and specificities from 68% to 92%, although some studies only included patients with elevated AFP levels [42–45]. In a prospective cohort study among 372 patients with HCV cirrhosis, of whom 34 developed HCC, AFP-L3 had a sensitivity and specificity of 37% and 92%, respectively, compared to 61% and 71% for AFP [44]. A recent large multicenter study demonstrated that AFP, at a cutoff of 10.9 ng/mL, is more sensitive for early-stage HCC than either of these two new biomarkers. AFP-L3 only had a sensitivity of 37% (95% CI 31–45%) for early-stage tumors and DCP had a sensitivity of 56% (95% CI 53–75%), whereas AFP had a sensitivity of 66% (95% CI 56–77%) [31].

Glypican 3, GP73, osteopontin, squamous cell carcinoma antigen, human hepatocyte growth factor, and insulin growth factor-1 are examples of other biomarkers that are currently being evaluated, but data are preliminary and require validation in large cohorts [46, 47]. Using proteomics-based approaches, more biomarkers are likely to be identified. A recent study determined that serum fibronectin is useful for the detection of early HCC and discrimination from cirrhosis with an AUC value of 0.832 [48]. A two-marker panel of fibronectin plus AFP demonstrated superior detection than AFP alone. Further studies, based on guidelines for biomarker development, are necessary to better evaluate the potential role of these biomarkers during surveillance.

For optimal detection of HCC, the most effective surveillance strategy may be a combination of several biomarkers. A model that included age, gender, AFP-L3, AFP, and DCP (GALAD) was evaluated in a multinational study with 2430 HCC patients and 4404 patients with chronic liver disease, demonstrating sensitivity exceeding 70% for overall HCC detection and exceeding 60% for early HCC detection [49]. Randomized studies that assess the clinical utility of the newer biomarkers and biomarker combinations for HCC surveillance are needed.

Surveillance Interval

HCC surveillance should be performed in at-risk individuals every 6 months. In a meta-analysis of prospective cohort studies evaluating the efficacy of surveillance tests for detecting HCC, ultrasound had a sensitivity of 70% in studies using a

6-month interval compared to a sensitivity of 50% in those with surveillance intervals between 6 and 12 months [50]. A retrospective analysis of a large prospectively maintained multicenter Italian database showed that patients who received surveillance every 6 months had tumors detected at an earlier stage and significantly better overall survival than patients receiving annual surveillance, even after correcting for lead-time bias [51]. The median corrected survival among the 510 patients in the 6-month surveillance group was 40.3 months, compared to 30 months in the 139 patients in the 12-month surveillance group ($p = 0.03$). A subsequent multicenter randomized controlled trial among 1340 patients with cirrhosis evaluated whether further shortening the surveillance interval to 3 months results in better detection of early-stage tumors and improves survival [32]. The majority of patients in both groups were detected at an early stage (79% vs. 71%, $p = 0.40$), and similar proportions received curative therapies (62% vs. 58%, $p = 0.88$). Furthermore, the 3-month surveillance group had a higher incidence of nonmalignant lesions, leading to a higher number of unnecessary recall procedures.

Surveillance Utilization

Despite more tumors detected at an early stage and the probable survival advantage, less than 20% of patients with cirrhosis undergo surveillance [52]. Among those receiving regular hepatology care by a specialist, surveillance rates are higher at 52%, but almost one-third receive inconsistent HCC surveillance, being evaluated less than once per year. HCC surveillance underuse can be attributed to several failures in the screening process including provider failure to identify liver disease, provider failure to identify the silent transition to cirrhosis, provider failure to order HCC surveillance, and patient failure to adhere with surveillance recommendations [53]. In a single-center study of patients with HCC, the most common reason for surveillance underuse was failure of providers to order HCC surveillance in patients with recognized cirrhosis. A survey study among primary care providers found several barriers to providers ordering HCC surveillance including insufficient knowledge regarding professional society guidelines for HCC surveillance, insufficient time in clinic, and competing clinical demands [54]. One study conducted at a safety-net health system suggested patients may also experience potential barriers to HCC surveillance including difficulty navigating the scheduling process, costs of surveillance tests, uncertainty where to complete surveillance, and transportation barriers; however, these results still require validation in other settings [55].

To address these lapses, models to improve surveillance have been proposed. For example, electronic medical record clinical reminders to perform surveillance significantly improved surveillance rates from 18.2% to 27.6% ($p < 0.001$) in a study among 2884 VA patients with cirrhosis who had not received HCC surveillance in the preceding 6 months [56]. A randomized study of surveillance among high-risk patients showed that a mailed outreach strategy that encouraged patients to undergo ultrasound screening with or without patient navigation also significantly improved

surveillance rates. One-time screening completion within 6 months was significantly higher in outreach/navigation (47.2%) and outreach-alone (44.5%) arms than usual care (24.3%) ($p < 0.001$ for both comparisons); however, screening rates did not significantly differ between outreach arms ($p = 0.25$) [57]. Similarly, HCC surveillance every 6 months over the 18-month study period was performed in 23.3% of outreach/navigation patients, 17.8% of outreach-alone patients, and 7.3% of usual care patients. HCC surveillance was significantly higher in both outreach groups than usual care ($p < 0.001$ for both) and higher for outreach/navigation than outreach-alone ($p = 0.02$). Despite improvements in surveillance rates in intervention studies to date, HCC surveillance in most intervention groups have remained disappointingly low, highlighting a need for more intensive interventions.

HCC Surveillance Cost-Effectiveness

The standard threshold for cost-effectiveness has been determined to be a maximal of $50,000 per quality-adjusted life year (QALY). HCC surveillance was determined to be cost-effective among HBV carriers when the incidence of HCC exceeds 0.2% per year. Surveillance with ultrasound and AFP has been demonstrated to be cost-effective in patients with compensated cirrhosis in several decision analysis models. Surveillance with biannual ultrasound and AFP in patients with Child-Turcotte-Pugh class A cirrhosis increases the mean life expectancy with cost-effectiveness ratios between $26,000 and $55,000 per QALY [58]. When a similar analysis was performed in patients with HCV cirrhosis, the cost-utility ratio was $26,689 per QALY [59]. Another study evaluating the cost-effectiveness of biannual AFP and ultrasound in HCV Child-Turcotte-Pugh class A cirrhosis revealed a cost-effectiveness ratio of $33,083 per QALY [60]. Individual risk-based personalized HCC surveillance strategies utilizing novel screening modalities such abbreviated MRI were reported to be substantially more cost-effective compared to the guideline-recommended uniform application of the biannual surveillance [61].

Staging

Patients diagnosed with HCC should be classified into prognostic groups based on the stage of disease to help inform clinical decision-making and provide the patient with the most appropriate treatment. Establishing a robust cancer staging system requires large numbers of patients who ideally remain untreated until death. This is typically not feasible in real life, so the performance of treatment stages may be impacted by discordant treatments that patients receive, even within the same stage. Though it is desirable to incorporate histology into prognostic systems, this is not included in most available staging systems given the large number of patients who were diagnosed solely based on imaging criteria. Because the attributes of HCC are

unique across different geographic regions, a universally applicable HCC staging system does not exist. Certain staging classifications work well in certain regions due to the local epidemiologic factors, such as the high HBV incidence in Asia and the increased likelihood of fatty liver disease in the United States due to a continued rise in obesity. There are also notable differences in the degree of liver dysfunction and aggressiveness of treatments, with higher rates of noncirrhotic HCC in Eastern populations and the use of resection even for multifocal tumors. In addition, staging HCC can be exceptionally difficult due to the interplay of underlying liver disease and cancer.

More than 11 different HCC staging systems exist [62–75]. Examples include Okuda, Cancer of the Liver Italian Program (CLIP), Japan Integrated Staging (JIS), the Barcelona Cancer Liver Cancer (BCLC) model, the Hong Kong Liver Cancer (HKLC) system, and ITA.LI.CA, as well as the conventional tumor, node, metastasis (TNM) system (Table 2.3). Most use parameters that reflect liver function, tumor status, and patient performance status – the three strongest prognostic factors for HCC. The CLIP scoring system which utilizes Child-Pugh staging and tumor burden considers AFP as a prognostic factor but does not incorporate patient symptoms [64]. The JIS and ITA.LI.CA models also recognize AFP as a prognostic factor [65, 68]. The TNM system relies on pathology and is only valid for resected tumors or transplanted livers and does not consider liver function. In addition, a prognostication model called BALAD that includes the biomarkers AFP-L3, AFP, and DCP in addition to bilirubin and albumin has been proposed and validated in a large international cohort [49]. Many of the HCC staging systems have limitations owing to their varying methodologies and populations used to develop the models. Although all can be utilized for patient prognostication, only BCLC and HKLC are linked to treatment recommendations.

Barcelona Cancer Liver Cancer (BCLC)

In the United States and Europe, the BCLC staging system is widely used (Fig. 2.2). The BCLC was developed based on several studies including the natural course of untreated HCC and survival after radical therapies [62]. The BCLC system first classified patients into four prognostic groups, A, B, C, and D, with stage 0 added later to recognize very-early-stage HCC. The BCLC system incorporates liver function (Child-Pugh class), tumor burden (number, size, vascular invasion, metastases), and patient performance status (Eastern Cooperative Oncology Group [ECOG] status). Whereas the BCLC system uses the Child-Pugh classification for liver function, both CLIP and JIS use the Model for End-stage Liver Disease (MELD) score.

Patients with BCLC stage 0 HCC, i.e., very early stage, have Child-Pugh class A cirrhosis, a single nodule that is less than 2 cm, and no cancer-related symptoms. BCLC stage A HCC, i.e., early stage, includes patients with single tumors of any size or up to three tumors that are smaller than 3 cm. Patients with BCLC stage A

Table 2.3 Hepatocellular carcinoma staging systems

Classification system	Components	Derivation cohort (year)
Okuda	Tumor burden Serum albumin Presence of ascites Total bilirubin	Multicenter Japanese Cohort (1985)
Cancer of the Liver Italian Program (CLIP)	Tumor morphology AFP Presence of portal vein invasion	Multicenter Italian Cohort (1998)
GRoupe d'Etude et de Traitement du Carcinoma Hépatocellulaire (GRETCH)	Presence of portal vein invasion AFP Total bilirubin Alkaline phosphatase Performance status	Multicenter French Cohort (1999)
Barcelona Cancer Liver Cancer (BCLC)	Tumor burden Child-Pugh score Functional status	Single Center European Cohort (1999)
Chinese University Prognostic Index (CUPI)	TNM staging AFP Total bilirubin Alkaline phosphatase Presence of ascites Presence of asymptomatic disease on presentation	Single Center Chinese Cohort (2002)
Japan Integrated Staging (JIS)	TNM staging Child-Pugh score	Multicenter Japanese Cohort (2003)
Tokyo Score	Tumor burden Serum albumin Total bilirubin	Single Center Japanese Cohort (2005)
Advanced Liver Cancer Prognostic System (ALCPS)	Presence of ascites Presence of abdominal pain Presence of weight loss Child-Pugh score Alkaline phosphatase Total bilirubin AFP Urea Tumor burden Presence of portal vein thrombosis Presence of lung metastases	Single Center Chinese Cohort (2008)
American Joint Committee on Cancer/TNM 7th edition	TNM staging Histologic grade Fibrosis score	Updated 2010
Taipei Integrated System	Tumor burden Child-Pugh score AFP	Single Center Taiwanese Cohort (2010)

(continued)

Table 2.3 (continued)

Classification system	Components	Derivation cohort (year)
Eastern Staging System	Presence of macro-/microvascular invasion Tumor burden Performance status Extrahepatic metastasis Serum albumin AST Total bilirubin Presence of cirrhosis	Single Center Chinese Cohort (2011)
Model to Estimate Survival in Ambulatory HCC patients (MESIAH)	Age MELD score Serum albumin Largest tumor diameter Number of tumors Presence of tumor vascular invasion Extrahepatic metastases AFP	Single Center US Cohort (2012)
Hong Kong Liver Cancer (HKLC)	Tumor burden Child-Pugh score Functional status	Single Center Chinese Cohort (2014)
ITA.LI.CA	Tumor burden Child-Pugh score Functional status AFP	Multicenter Italian Cohort (2016)
NIACE (tumor Nodularity, Infiltrative nature of the tumor, AFP level, Child-Pugh class, and ECOG)	Tumor burden AFP Child-Pugh score Functional status	Multicenter French Cohort (2016)

HCC have preserved liver function (Child-Pugh class A and B) and no cancer-related symptoms. Patients classified as BCLC stage 0 or A are eligible for curative therapies, such as surgical resection, liver transplantation, and percutaneous ablation, and typically achieve 5-year survival rates greater than 70%. Patients with BCLC stage B HCC, i.e., intermediate stage, are asymptomatic and have preserved liver function (Child-Pugh class A and B) but have multinodular tumors exceeding BCLC stage A without vascular invasion or extrahepatic spread. Treatment of BCLC stage B patients usually entails locoregional therapy, such as transarterial chemoembolization (TACE) or transarterial radioembolization (TARE). The median survival of untreated stage B patients is approximately 2 years, although this differs widely between BCLC stage B patients depending on tumor burden and Child-Pugh class. Patients with advanced HCC are classified as BCLC stage C and have tumors with vascular invasion and/or extrahepatic spread with or without cancer-related symptoms (ECOG of 1–2). Patients with advanced HCC are typically treated with systemic therapies and have a median survival of approximately 1 year. Terminal-stage HCC, i.e., BCLC stage D, is treated symptomatically with best supportive care given severe symptoms (ECOG 3–4) and/or poor liver function (Child-Pugh C) pre-

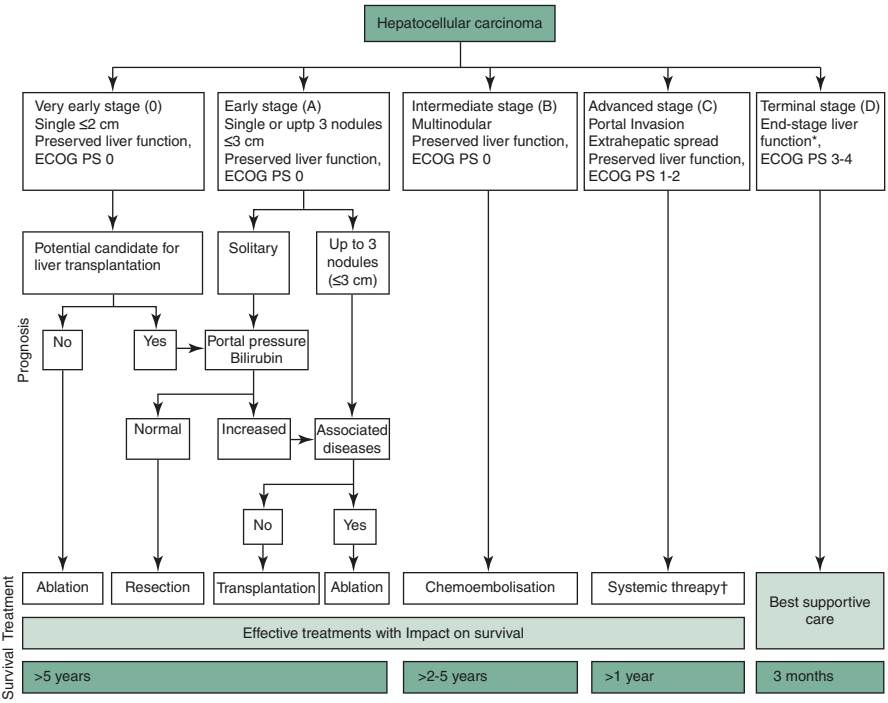

Fig. 2.2 Barcelona Clinic Liver Cancer (BCLC) staging system

cluding a benefit from other HCC-directed therapies. One criticism of the BCLC is heterogeneity of prognosis within BCLC stage B and BCLC stage C and failure to incorporate more aggressive therapies for selected patients who may benefit. Specifically, many Asian centers offer surgical resection in selected patients with BCLC stage B (limited multifocal HCC) or stage C (small intrahepatic vascular invasion) HCC, whereas the BCLC treatment algorithms do not mention this option. Another criticism includes the imprecise use of the ECOG performance status, which could be influenced by hepatic function, cancer symptoms, or nonliver-related conditions.

The prognostic ability of the BCLC has been validated in European, American, and Asian populations. In a study comparing the prognostic ability of seven staging systems, the BCLC was found to have the best independent predictive power for survival [76]. The median survival for patients with BCLC stage D tumors was approximately 5 months, which was significantly shorter than the 10-month median survival for those with BCLC stage C tumors ($p = 0.01$). Patients with BCLC stage B tumors had a median survival of approximately 27 months ($p = 0.04$ vs. BCLC stage C tumors) and BCLC stage A patients had a median survival exceeding 4 years ($p < 0.001$ vs. BCLC stage B). Similarly, the BCLC staging system was validated in a prospective assessment of 195 Italian patients with HCC and demonstrated to have the best predictive power compared with other staging systems [77]. However, the validity of the BCLC staging system will need to be reevaluated in the future and

compared to newer staging systems, particularly given the progress in both risk stratifications and treatment.

Hong Kong Liver Cancer (HKLC)

The HKLC staging system, like the BCLC, is useful for guiding treatment algorithms as well as for patient prognostication (Fig. 2.3). This system was created based on data collected retrospectively from a large Hong Kong cohort of 3856 patients with HCC treated between 1995 and 2008 [66]. Similar to the BCLC system, the HKLC system also incorporates Child-Pugh class, ECOG status, and tumor burden. Patients are first divided according to ECOG status (0–1 vs. 2–4) and Child-Pugh class (A–B vs. C) and then stratified according to presence or absence of extravascular invasion/metastasis and tumor burden, yielding a total of nine possible stages, to identify best treatment options. Importantly, the HKLC system identified subsets of patients classified as BCLC B and BCLC C for which more aggressive treatments led to improved patient survival. Hypothetical Kaplan-Meier survival curves showed the HKLC treatment algorithm yielded better survival outcomes (median overall survival: HKLC, 16.6 months, BCLC, 8.9 months) [66]. A caveat with using a more complex system, such as HKLC, is its implementation in the clinic, as many clinicians find the BCLC more user-friendly. As more variables are included in a staging system, using it efficiently at bedside becomes increasingly difficult. Further, the Kaplan-Meier curves do not demonstrate discrimination of survival between some advanced stages (e.g., stages 3b to 5a).

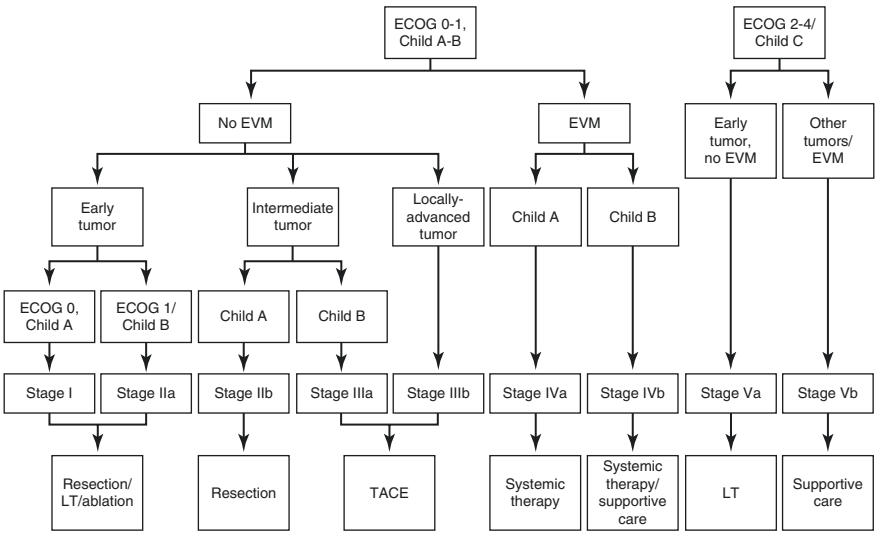

Fig. 2.3 Hong Kong Liver Cancer (HKLC) staging system

Although the HKLC is more aggressive in treatment recommendations, it may not necessarily do a better job of predicting survival than other staging systems. External validation in Western populations is particularly important given the HKLC system was developed in an Eastern patient population with the majority having underlying HBV-related liver disease. The prognostic performance of the HKLC system was evaluated in a North American cohort of 881 patients with HCC undergoing intra-arterial therapy. Although both BCLC and HKLC predict patient survival with high certainty, the HKLC-5 system outperformed the BCLC system in terms of survival separation, calibration, and discrimination (c-statistic 0.707 for HKLC vs. 0.643 for BCLC) [78]. In a European population, the HKLC treatment algorithm resulted in significantly more patients assigned to curative therapy than the BCLC algorithm; however, the BCLC system outperformed the HKLC classification in survival prediction [79].

Additional HCC Staging Systems

A nomogram based on the BCLC model was recently proposed to address the diverse prognosis of subgroups of BCLC stages and improve its prognostic value [80]. The nomogram generated a c-statistic of 0.766 (95% CI: 0.686–0.808) in the derivation cohort and 0.775 (0.607–0.909) in the validation cohort, both of which were Asian populations. The BCLC-based nomogram yielded a better survival distribution, especially for patients staged BCLC C and D when compared with published results. Since the BCLC-based nomogram uses the same clinical parameters as BCLC, tumor burden, performance status, and liver disease, and assigns a score from 0 to 26, the authors suggested that it would be easy to use by clinicians. However, external validation in a French group concluded that the system was limited by its complexity and absence of linkage to treatment strategies. The BCLC-based nomogram has recently undergone further refinements using primary treatment as a factor in model construction [81]. The revised treatment-integrated nomogram showed larger linear trend x^2 and likelihood ratio x^2 values, and similar c-statistic (0.774) as the original nomogram.

The ITA.LI.CA stratifies patients with HCC into six groups and includes AFP as a prognostic factor [68]. The ITA.LI.CA model was developed in an Italian patient cohort of mostly HCV carriers, and externally validated in an Asian cohort of mostly HBV carriers. The core component of ITA.LI.CA incorporates a tumor staging which has stages 0, A, B, and C. Stage 0 includes tumors $\leq$2 cm; stage A involves tumors within Milan criteria for transplantation (single lesion not more than 5 cm, up to three lesions, none more than 3 cm); stage B is divided into B1, B2, and B3 with B3 according to size and number cutoffs of 5 cm and three lesions, respectively; and stage C includes patients with extrahepatic vascular involvement or distant metastasis. In addition to tumor burden, the ITA.LI.CA staging system also incorporates Child-Pugh class, ECOG performance status, and AFP to create a final 0–13-point score. In comparison with other staging systems, including HKLC and

BCLC, there is some data to show that ITA.LI.CA showed significantly better prognostication.

The Model to Estimate Survival in Ambulatory HCC patients (MESIAH) scoring system utilizes the MELD parameter and was developed in an American cohort and externally validated in a Korean cohort [69]. The c-statistic in the derivation cohort with MESIAH was 0.77, which was higher than for CLIP (0.70), JIS (0.70), and BCLC (0.71). The MESIAH scoring system performed equally well in patients with cirrhosis (0.77) as in patients without cirrhosis (0.78).

The NIACE (tumor Nodularity, Infiltrative nature of the tumor, AFP level, Child-Pugh class, and ECOG) score was also developed to help refine prognoses within the BCLC A, B, and C groups. When used in combination with the BCLC-based nomogram, NIACE demonstrated the best predictive accuracy for overall survival in a French cohort of 1102 patients compared with the BCLC, HKLC, and CLIP systems [82]. The NIACE score significantly differentiated survival times in BCLC A patients treated with surgery and BCLC B patients treated with chemoembolization [83]. Some have suggested that the best strategy may encompass utilization of a staging system such as BCLC in combination with a complementary scoring system like NIACE.

The staging system with the most clinical utility is yet to be determined as investigations that have compared different staging systems have generated differing conclusions. At this time, although the BCLC staging system for HCC has its limitations, it is relatively easy to categorize patients and thus remains the most widely used staging system in the United States and Europe.

Conclusion

The rising incidence of HCC worldwide is a major health concern with high mortality rates. Although the benefits of HCC surveillance are well-documented, surveillance implementation and effectiveness in clinical practice remains suboptimal so most HCC patients present at an advanced stage with limited treatment options. Although there is not a universally accepted staging system, the BCLC remains the most widely used given its balance of accuracy and ease of use. Staging systems are continuously being developed and restructured to aid in prognostication, guide clinical decision-making, and improve overall survival in this challenging and heterogeneous patient population.

References

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264–73.e1. https://doi.org/10.1053/j.gastro.2011.12.061.
2. Khalaf N, Ying J, Mittal S, Temple S, Kanwal F, Davila J, et al. Natural history of untreated hepatocellular carcinoma in a US cohort and the role of cancer surveillance. Clin Gastroenterol Hepatol. 2017;15(2):273–81.e1. https://doi.org/10.1016/j.cgh.2016.07.033.

3. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018;69(1):182–236. https://doi.org/10.1016/j.jhep.2018.03.019.
4. Covey AM. Hepatocellular carcinoma: updates to screening and diagnosis. J Natl Compr Cancer Netw: JNCCN. 2018;16(5s):663–5. https://doi.org/10.6004/jnccn.2018.0052.
5. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67(1):358–80. https://doi.org/10.1002/hep.29086.
6. Yang B, Zhang B, Xu Y, Wang W, Shen Y, Zhang A, et al. Prospective study of early detection for primary liver cancer. J Cancer Res Clin Oncol. 1997;123(6):357–60.
7. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417–22. https://doi.org/10.1007/s00432-004-0552-0.
8. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. https://doi.org/10.1371/journal.pmed.1001624.
9. Kansagara D, Papak J, Pasha AS, O'Neil M, Freeman M, Relevo R, et al. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. Ann Intern Med. 2014;161(4):261–9. https://doi.org/10.7326/M14-0558.
10. Trevisani F, De Notariis S, Rapaccini G, Farinati F, Benvegnu L, Zoli M, et al. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). Am J Gastroenterol. 2002;97(3):734–44. https://doi.org/10.1111/j.1572-0241.2002.05557.x.
11. El-Serag HB, Kramer JR, Chen GJ, Duan Z, Richardson PA, Davila JA. Effectiveness of AFP and ultrasound tests on hepatocellular carcinoma mortality in HCV-infected patients in the USA. Gut. 2011;60(7):992–7. https://doi.org/10.1136/gut.2010.230508.
12. van Meer S, de Man RA, Coenraad MJ, Sprengers D, van Nieuwkerk KM, Klumpen HJ, et al. Surveillance for hepatocellular carcinoma is associated with increased survival: results from a large cohort in the Netherlands. J Hepatol. 2015;63(5):1156–63. https://doi.org/10.1016/j.jhep.2015.06.012.
13. Singal AG, Mittal S, Yerokun OA, Ahn C, Marrero JA, Yopp AC, et al. Hepatocellular carcinoma screening associated with early tumor detection and improved survival among patients with cirrhosis in the US. Am J Med. 2017;130(9):1099–106 e1. https://doi.org/10.1016/j.amjmed.2017.01.021.
14. Costentin C, Layese R, Bourcier V, Cagnot C, Marcellin P, Guyader D, et al. Compliance with hepatocellular carcinoma surveillance guidelines associated with increased lead-time adjusted survival of patients with compensated viral cirrhosis. Gastroenterology. 2018. https://doi.org/10.1053/j.gastro.2018.04.027.
15. Harris RP, Sheridan SL, Lewis CL, Barclay C, Vu MB, Kistler CE, et al. The harms of screening: a proposed taxonomy and application to lung cancer screening. JAMA Intern Med. 2014;174(2):281–5. https://doi.org/10.1001/jamainternmed.2013.12745.
16. Taylor EJ, Jones RL, Guthrie JA, Rowe IA. Modeling the benefits and harms of surveillance for hepatocellular carcinoma: information to support informed choices. Hepatology (Baltimore, Md). 2017;66(5):1546–55. https://doi.org/10.1002/hep.29315.
17. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, Parikh ND, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. Hepatology. 2017;65(4):1196–205. https://doi.org/10.1002/hep.28895.
18. Zhang B, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. J Med Screen. 1999;6(2):108–10. https://doi.org/10.1136/jms.6.2.108.
19. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. Gastroenterology. 2010;138(2):493–502. https://doi.org/10.1053/j.gastro.2009.10.031.

20. Singal AG, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. Cancer Epidemiol Biomark Prev. 2012;21(5):793–9. https://doi.org/10.1158/1055-9965.EPI-11-1005.
21. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. Gastroenterology. 2018;154(6):1706–18 e1. https://doi.org/10.1053/j.gastro.2018.01.064.
22. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. Aliment Pharmacol Ther. 2017;45(1):169–77. https://doi.org/10.1111/apt.13841.
23. Del Poggio P, Olmi S, Ciccarese F, Di Marco M, Rapaccini GL, Benvegnu L, et al. Factors that affect efficacy of ultrasound surveillance for early stage hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014;12(11):1927–33 e2. https://doi.org/10.1016/j.cgh.2014.02.025.
24. Singal AG, Nehra M, Adams-Huet B, Yopp AC, Tiro JA, Marrero JA, et al. Detection of hepatocellular carcinoma at advanced stages among patients in the HALT-C trial: where did surveillance fail? Am J Gastroenterol. 2013;108(3):425–32. https://doi.org/10.1038/ajg.2012.449.
25. Pocha C, Dieperink E, McMaken KA, Knott A, Thuras P, Ho SB. Surveillance for hepatocellular cancer with ultrasonography vs. computed tomography – a randomised study. Aliment Pharmacol Ther. 2013;38(3):303–12. https://doi.org/10.1111/apt.12370.
26. Van Thiel DH, Yong S, Li SD, Kennedy M, Brems J. The development of de novo hepatocellular carcinoma in patients on a liver transplant list: frequency, size, and assessment of current screening methods. Liver Transpl. 2004;10(5):631–7. https://doi.org/10.1002/lt.20120.
27. Kim SY, An J, Lim YS, Han S, Lee JY, Byun JH, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. JAMA Oncol. 2017;3(4):456–63. https://doi.org/10.1001/jamaoncol.2016.3147.
28. Shah TU, Semelka RC, Pamuklar E, Firat Z, Gerber RD, Shrestha R, et al. The risk of hepatocellular carcinoma in cirrhotic patients with small liver nodules on MRI. Am J Gastroenterol. 2006;101(3):533–40. https://doi.org/10.1111/j.1572-0241.2006.00450.x.
29. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol. 2001;34(4):570–5.
30. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. a systematic review and critical analysis. Ann Intern Med. 2003;139(1):46–50.
31. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009;137(1):110–8. https://doi.org/10.1053/j.gastro.2009.04.005.
32. Trinchet JC, Chaffaut C, Bourcier V, Degos F, Henrion J, Fontaine H, et al. Ultrasonographic surveillance of hepatocellular carcinoma in cirrhosis: a randomized trial comparing 3- and 6-month periodicities. Hepatology. 2011;54(6):1987–97. https://doi.org/10.1002/hep.24545.
33. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43(3):434–41.
34. Gopal P, Yopp AC, Waljee AK, Chiang J, Nehra M, Kandunoori P, et al. Factors that affect accuracy of alpha-fetoprotein test in detection of hepatocellular carcinoma in patients with cirrhosis. Clinical gastroenterology and hepatology : the official clinical practice. J Am Gastroenterol Assoc. 2014;12(5):870–7. https://doi.org/10.1016/j.cgh.2013.09.053.
35. El-Serag HB, Kanwal F, Davila JA, Kramer J, Richardson P. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. Gastroenterology. 2014;146(5):1249–55 e1. https://doi.org/10.1053/j.gastro.2014.01.045.
36. Wang M, Devarajan K, Singal AG, Marrero JA, Dai J, Feng Z, et al. The Doylestown algorithm: a test to improve the performance of AFP in the detection of hepatocellular carcinoma. Cancer Prev Res (Phila). 2016;9(2):172–9. https://doi.org/10.1158/1940-6207.CAPR-15-0186.

37. Lee E, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of alpha-fetoprotein, over time. Clin Gastroenterol Hepatol. 2013;11(4):437–40. https://doi.org/10.1016/j.cgh.2012.11.029.
38. Tayob N, Stingo F, Do KA, Lok ASF, Feng Z. A Bayesian screening approach for hepatocellular carcinoma using multiple longitudinal biomarkers. Biometrics. 2018;74(1):249–59. https://doi.org/10.1111/biom.12717.
39. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. Hepato-Gastroenterology. 2002;49(43):235–8.
40. Ishii M, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, et al. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. Am J Gastroenterol. 2000;95(4):1036–40. https://doi.org/10.1111/j.1572-0241.2000.01978.x.
41. Izuno K, Fujiyama S, Yamasaki K, Sato M, Sato T. Early detection of hepatocellular carcinoma associated with cirrhosis by combined assay of des-gamma-carboxy prothrombin and alpha-fetoprotein: a prospective study. Hepato-Gastroenterology. 1995;42(4):387–93.
42. Shiraki K, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. Hepatology (Baltimore, Md). 1995;22(3):802–7.
43. Sterling RK, Jeffers L, Gordon F, Sherman M, Venook AP, Reddy KR, et al. Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. Am J Gastroenterol. 2007;102(10):2196–205. https://doi.org/10.1111/j.1572-0241.2007.01405.x.
44. Sterling RK, Jeffers L, Gordon F, Venook AP, Reddy KR, Satomura S, et al. Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2009;7(1):104–13. https://doi.org/10.1016/j.cgh.2008.08.041.
45. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y, et al. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. Gastroenterology. 1990;99(2):508–18.
46. Beneduce L, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnu L, et al. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. Cancer. 2005;103(12):2558–65. https://doi.org/10.1002/cncr.21106.
47. Yamagami H, Moriyama M, Tanaka N, Arakawa Y. Detection of serum and intrahepatic human hepatocyte growth factor in patients with type C liver diseases. Intervirology. 2001;44(1):36–42. https://doi.org/10.1159/000050028.
48. Kim H, Park J, Kim Y, Sohn A, Yeo I, Jong Yu S, et al. Serum fibronectin distinguishes the early stages of hepatocellular carcinoma. Sci Rep. 2017;7(1):9449. https://doi.org/10.1038/s41598-017-09691-3.
49. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016;14(6):875–86.e6. https://doi.org/10.1016/j.cgh.2015.12.042.
50. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30(1):37–47. https://doi.org/10.1111/j.1365-2036.2009.04014.x.
51. Santi V, Trevisani F, Gramenzi A, Grignaschi A, Mirici-Cappa F, Del Poggio P, et al. Semiannual surveillance is superior to annual surveillance for the detection of early hepatocellular carcinoma and patient survival. J Hepatol. 2010;53(2):291–7. https://doi.org/10.1016/j.jhep.2010.03.010.
52. Singal AG, Yopp A, C SS, Packer M, Lee WM, Tiro JA. Utilization of hepatocellular carcinoma surveillance among American patients: a systematic review. J Gen Intern Med. 2012;27(7):861–7. https://doi.org/10.1007/s11606-011-1952-x.
53. Singal AG, Yopp AC, Gupta S, Skinner CS, Halm EA, Okolo E, et al. Failure rates in the hepatocellular carcinoma surveillance process. Cancer Prev Res (Phila). 2012;5(9):1124–30. https://doi.org/10.1158/1940-6207.CAPR-12-0046.

54. Dalton-Fitzgerald E, Tiro J, Kandunoori P, Halm EA, Yopp A, Singal AG. Practice patterns and attitudes of primary care providers and barriers to surveillance of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2015;13(4):791–8 e1. https://doi.org/10.1016/j.cgh.2014.06.031.
55. Farvardin S, Patel J, Khambaty M, Yerokun OA, Mok H, Tiro JA, et al. Patient-reported barriers are associated with lower hepatocellular carcinoma surveillance rates in patients with cirrhosis. Hepatology (Baltimore, Md). 2017;65(3):875–84. https://doi.org/10.1002/hep.28770.
56. Beste LA, Ioannou GN, Yang Y, Chang MF, Ross D, Dominitz JA. Improved surveillance for hepatocellular carcinoma with a primary care-oriented clinical reminder. Clin Gastroenterol Hepatol. 2015;13(1):172–9. https://doi.org/10.1016/j.cgh.2014.04.033.
57. Singal AG, Tiro JA, Marrero JA, McCallister K, Mejias C, Adamson B, et al. Mailed outreach program increases ultrasound screening of patients with cirrhosis for hepatocellular carcinoma. Gastroenterology. 2017;152(3):608–15.e4. https://doi.org/10.1053/j.gastro.2016.10.042.
58. Sarasin FP, Giostra E, Hadengue A. Cost-effectiveness of screening for detection of small hepatocellular carcinoma in western patients with Child-Pugh class A cirrhosis. Am J Med. 1996;101(4):422–34. https://doi.org/10.1016/S0002-9343(96)00197-0.
59. Arguedas MR, Chen VK, Eloubeidi MA, Fallon MB. Screening for hepatocellular carcinoma in patients with hepatitis C cirrhosis: a cost-utility analysis. Am J Gastroenterol. 2003;98(3):679–90.
60. Lin OS, Keeffe EB, Sanders GD, Owens DK. Cost-effectiveness of screening for hepatocellular carcinoma in patients with cirrhosis due to chronic hepatitis C. Aliment Pharmacol Ther. 2004;19(11):1159–72. https://doi.org/10.1111/j.1365-2036.2004.01963.x.
61. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, Besa C, et al. Cost-effectiveness of risk score-stratified hepatocellular carcinoma screening in patients with cirrhosis. Clin Transl Gastroenterol. 2017;8(6):e101. https://doi.org/10.1038/ctg.2017.26.
62. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19(3):329–38. https://doi.org/10.1055/s-2007-1007122.
63. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985;56(4):918–28.
64. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology (Baltimore, Md). 1998;28(3):751–5. https://doi.org/10.1002/hep.510280322.
65. Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). J Gastroenterol. 2003;38(3):207–15. https://doi.org/10.1007/s005350300038.
66. Yau T, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. Gastroenterology. 2014;146(7):1691–700.e3. https://doi.org/10.1053/j.gastro.2014.02.032.
67. Yau T, Yao TJ, Chan P, Ng K, Fan ST, Poon RT. A new prognostic score system in patients with advanced hepatocellular carcinoma not amendable to locoregional therapy: implication for patient selection in systemic therapy trials. Cancer. 2008;113(10):2742–51. https://doi.org/10.1002/cncr.23878.
68. Farinati F, Vitale A, Spolverato G, Pawlik TM, Huo TL, Lee YH, et al. Development and validation of a new prognostic system for patients with hepatocellular carcinoma. PLoS Med. 2016;13(4):e1002006. https://doi.org/10.1371/journal.pmed.1002006.
69. Yang JD, Kim WR, Park KW, Chaiteerakij R, Kim B, Sanderson SO, et al. Model to estimate survival in ambulatory patients with hepatocellular carcinoma. Hepatology (Baltimore, Md). 2012;56(2):614–21. https://doi.org/10.1002/hep.25680.
70. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. Cancer. 2002;94(6):1760–9.

71. Adhoute X, Penaranda G, Raoul JL, Blanc JF, Edeline J, Conroy G, et al. Prognosis of advanced hepatocellular carcinoma: a new stratification of Barcelona Clinic Liver Cancer stage C: results from a French multicenter study. Eur J Gastroenterol Hepatol. 2016;28(4):433–40. https://doi.org/10.1097/meg.0000000000000558.
72. Tateishi R, Yoshida H, Shiina S, Imamura H, Hasegawa K, Teratani T, et al. Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. Gut. 2005;54(3):419–25. https://doi.org/10.1136/gut.2003.035055.
73. Hsu CY, Huang YH, Hsia CY, Su CW, Lin HC, Loong CC, et al. A new prognostic model for hepatocellular carcinoma based on total tumor volume: the Taipei Integrated Scoring System. J Hepatol. 2010;53(1):108–17. https://doi.org/10.1016/j.jhep.2010.01.038.
74. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. J Hepatol. 1999;31(1):133–41.
75. Yang T, Zhang J, Lu JH, Yang LQ, Yang GS, Wu MC, et al. A new staging system for resectable hepatocellular carcinoma: comparison with six existing staging systems in a large Chinese cohort. J Cancer Res Clin Oncol. 2011;137(5):739–50. https://doi.org/10.1007/s00432-010-0935-3.
76. Marrero JA, Fontana RJ, Barrat A, Askari F, Conjeevaram HS, Su GL, et al. Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. Hepatology (Baltimore, Md). 2005;41(4):707–16. https://doi.org/10.1002/hep.20636.
77. Cillo U, Vitale A, Grigoletto F, Farinati F, Brolese A, Zanus G, et al. Prospective validation of the Barcelona Clinic Liver Cancer staging system. J Hepatol. 2006;44(4):723–31. https://doi.org/10.1016/j.jhep.2005.12.015.
78. Sohn JH, Duran R, Zhao Y, Fleckenstein F, Chapiro J, Sahu S, et al. Validation of the Hong Kong Liver Cancer staging system in determining prognosis of the North American patients following intra-arterial therapy. Clin Gastroenterol Hepatol. 2017;15(5):746–55.e4. https://doi.org/10.1016/j.cgh.2016.10.036.
79. Kolly P, Reeves H, Sangro B, Knopfli M, Candinas D, Dufour JF. Assessment of the Hong Kong Liver Cancer staging system in Europe. Liver Int. 2016;36(6):911–7. https://doi.org/10.1111/liv.13045.
80. Hsu CY, Liu PH, Hsia CY, Lee YH, Al Juboori A, Lee RC, et al. Nomogram of the Barcelona Clinic Liver Cancer system for individual prognostic prediction in hepatocellular carcinoma. Liver Int. 2016;36(10):1498–506. https://doi.org/10.1111/liv.13114.
81. Hsu CY, Liu PH, Hsia CY, Lee YH, Nagaria TS, Lee RC, et al. A new treatment-integrated prognostic nomogram of the Barcelona Clinic Liver Cancer system for hepatocellular carcinoma. Sci Rep. 2017;7(1):7914. https://doi.org/10.1038/s41598-017-08382-3.
82. Adhoute X, Penaranda G, Raoul JL, Edeline J, Blanc JF, Pol B, et al. Barcelona Clinic Liver Cancer nomogram and others staging/scoring systems in a French hepatocellular carcinoma cohort. World J Gastroenterol. 2017;23(14):2545–55. https://doi.org/10.3748/wjg.v23.i14.2545.
83. Adhoute X, Penaranda G, Raoul JL, Bollon E, Pol B, Letreut YP, et al. NIACE score for hepatocellular carcinoma patients treated by surgery or transarterial chemoembolization. Eur J Gastroenterol Hepatol. 2017;29(6):706–15. https://doi.org/10.1097/meg.0000000000000852.

Chapter 3
Changing Epidemiology of Hepatocellular Carcinoma and Role of Surveillance

Yueran Zhuo, Qiushi Chen, and Jagpreet Chhatwal

Introduction

Hepatocellular carcinoma (HCC) is responsible for 80–90% of primary liver cancer cases and is the third most common cause of cancer deaths worldwide [1, 2]. In the United States, HCC is the fastest growing cause of cancer deaths. While the overall cancer death rate has declined by 18% in the last two decades, HCC-related mortality has increased by 40% during the same period (Fig. 3.1 shows mortality in men, who are 4–8 times more likely than women to develop HCC) [3]. In addition, HCC incidence has increased threefold between 1975 and 2009, and the upward trend continues (Fig. 3.2) [4]. The rising burden of HCC was also highlighted by the *2015 Annual Report on the Status of Cancer* [5]. Common risk factors for HCC include hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, heavy alcohol use leading to alcoholic liver disease (ALD), and nonalcoholic steatohepatitis (NASH).

Survival after diagnosis of HCC is worse than that of almost every other major form of cancer, including the lung, esophagus, and stomach [5]. While patients with advanced HCC have a median survival of less than 1 year, patients with early HCC who receive potentially curative therapy such as liver transplantation or resection achieve 5-year survival rates near 70%. Early diagnosis, therefore, is critical to improved survival. However, unlike other major cancers such as breast, prostate, and colorectal cancers, surveillance for HCC has been underutilized in practice [6]. While ultrasound-based surveillance with or without α-fetoprotein (AFP) is

Y. Zhuo · J. Chhatwal (✉)
Institute for Technology Assessment, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: jagchhatwal@mgh.harvard.edu

Q. Chen
Harold and Inge Marcus Department of Industrial and Manufacturing Engineering, Pennsylvania State University, University Park, PA, USA

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_3

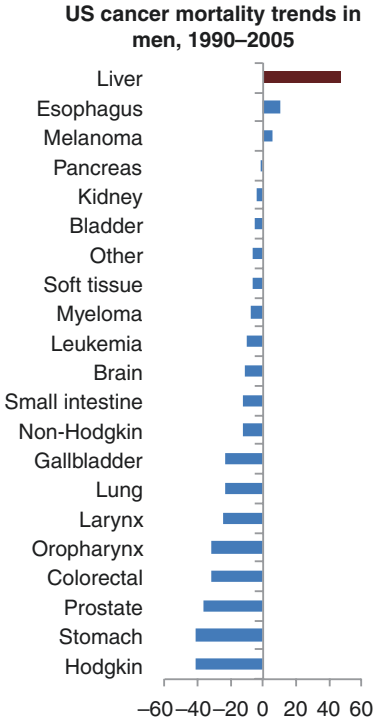

Fig. 3.1 Trend of cancer mortality change in men in the United States

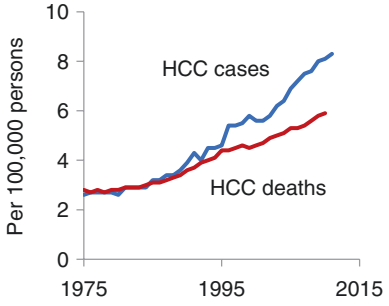

Fig. 3.2 Trend of HCC incidence between 1975 and 2015

recommended in high-risk individuals (e.g., cirrhosis), surveillance failures are common, and the majority of patients with HCC are diagnosed at an advanced stage [7]. Furthermore, fewer than 20% of patients with cirrhosis receive regular surveillance [8, 9]. Another key factor that distinguishes HCC from most other cancers is that it primarily occurs in the setting of end-stage liver failure, which severely limits treatment options such as curative resection as well as palliative locoregional therapy. Liver transplantation is a life-saving modality but is a highly restricted resource with long waiting lists.

HCC Incidence and Mortality Trends

According to the 2015 Global Burden of Disease Study [10], there were 854,000 incident cases of liver cancer and 810,000 deaths globally in 2015. According to the study, HBV is the most prevalent cause of HCC, accounting for 33% of all the HCC deaths globally. Alcohol is the second most common cause of HCC, accounting for 30% of all HCC deaths. HCV accounted for 21% of all HCC deaths and other causes including NASH accounted for the remaining 16%. These distributions of HCC-led death etiologies vary across different geographical regions (Table 3.1). The study also noted that the etiologies of HCC vary substantially across different countries and regions. The study also reported increasing trends in HCC incidence across all etiology groups from 1990 to 2015—HBV-related HCC incidence rates increased by 42%, HCV-related HCC incidence by 114%, ALD-related HCC incidence by 109%, and other causes by 56%. The increasing rates in HCC incidence occurred primarily due to population growth and aging of population.

Table 3.1 Contribution of hepatitis B, hepatitis C, alcohol, and other causes on absolute liver cancer deaths, both sexes, globally and by region, 2015

	Contributor of HCC-related death			
	Alcohol (%)	HBV (%)	HCV (%)	Other (including NASH) (%)
Australasia	39	9	39	13
Caribbean	25	26	30	19
Central Asia	20	30	37	13
Central Europe	46	15	29	10
Central Latin America	27	8	47	18
Central sub-Saharan Africa	29	20	37	13
East Asia	32	41	9	18
Eastern Europe	53	15	24	8
Eastern sub-Saharan Africa	32	26	28	14
High-income Asia Pacific	18	22	55	6
High-income North America	37	9	31	23
North Africa and Middle East	13	27	44	16
Oceania	16	38	19	27
South Asia	18	38	25	19
Southeast Asia	31	26	22	21
Southern Latin America	42	6	41	11
Southern sub-Saharan Africa	40	29	20	11
Tropical Latin America	32	20	35	13
Western Europe	32	13	44	10
Western sub-Saharan Africa	29	45	11	15

Adapted from [10]

Table 3.2 Etiology distribution of HCC in selective Asian countries

	HBV (%)	HCV (%)	Alcohol (%)	Others (%)	Unknown (%)
China	70	–	–	–	30
Hong Kong	80	6	5	9	–
South Korea	63	14	11	7	5
Japan	16	70	–	14	
Taiwan	66	32	–	–	2
Philippines	67	3	10	20	–
Singapore	57	2	11	28	2
Malaysia	85	2	4	–	9
Thailand	80	15	–	–	5
India	70	12	16	–	2

Adapted from [12]

Asia presented very unique HCC etiologies among all the populations in the world, while a great majority of the HCC occurred in Asia.

The burden of HCC is in particular high in Asia. In 2018, around 47% of the 841,000 new hepatocellular carcinoma cases occurred in China alone [11]. The etiology of HCC in selected Asian countries is summarized in Table 3.2 [12]. Unlike other parts of the world, the leading cause of HCC in Asia has been HBV [13]. The only exception is Japan, where HCV is the leading cause for HCC. Since most Asian countries started implementing nationwide hepatitis B immunization programs in the last couple of decades, the seroprevalence of HBV among children and young adults has declined significantly in these countries [14–16]. It is expected that HBV-associated HCC incidence will decrease substantially in younger generations in these Asian countries. In addition, advanced HBV treatment methods have also significantly reduced the risk of HCC among HBV-infected people [17, 18]. However, other HCC-leading causes are on the rise, while HBV is on the decline. Specifically, the rapid socioeconomic development in many Asian countries has caused lifestyles and dietary patterns that lead to more cases of NASH-related HCC [19]. With the controlling of HBV in most Asian countries and HCV epidemics in Japan, NASH is gaining prominence and will likely become the new leading cause of HCC in the years to come.

In the United States, the HCC incidence has increased sharply in the past a couple of decades. A population-based descriptive study showed that the age-adjusted incidence rates of HCC increased by 52% from year 2000 to 2012 [20]. The rising HCC incidence is primarily related to an aging population with chronic HCV infection [21], but this is expected to change in the near future because of the availability of highly effective HCV treatments [22, 23]. NASH-related HCC incidence, on the other hand, is rising because of obesity and nonalcoholic fatty liver disease (NAFLD) [24, 25]. The incidence of HBV-led HCC is likely to remain steady [26]. The mortality rates due to HCC have also increased over the past a couple of decades. As shown by an observational study [27], from year 1999 to 2006, the annual HCC-related deaths double to 11,073, particularly highlighting alcohol as the reason for HCC-related deaths among young people between ages 25 and 34.

Table 3.3 Model-projected HCC incidence and associated deaths in 16 countries from 2013 to 2030 [28]

	HCC incidence		HCC-related deaths	
	Base case	Percentage increase by 2030	Base case	Percentage increase by 2030
Australia	590	245%	530	230%
Austria	110	35%	100	25%
Belgium	300	110%	290	95%
Brazil	9710	95%	9000	85%
Canada	730	190%	720	150%
Czech Republic	90	85%	80	90%
Denmark	90	140%	80	130%
Egypt	16,050	15%	32,950	10%
England	410	125%	390	100%
France	1790		1630	76%
Germany	1530	10%	1300	10%
Portugal	1150	80%	890	90%
Spain	2210	105%	1940	95%
Sweden	270	10%	170	1%
Switzerland	400	85%	380	70%
Turkey	2230	70%	2020	70%

Future trends in HCC-related incidence and mortality and underlying etiology can be projected using decision-analytic modeling. A modeling study projected current and future impact of HCV disease burden in 16 countries [28]. It projected a 245% increase in HCC incidence rates from 2013 to 2030 in these countries. Similarly, the number of liver-related death is projected to increase up to 230% in all countries except for Sweden. Table 3.3 summarizes the model projected HCC and LRD trend in these 16 countries. However, with aggressive HCV screening along with unrestricted access to new antivirals, HCC incidence can be reduced substantially.

In the United States, HCV disease burden and related HCC burden in the era of Direct-acting antivirals (DAAs) have been projected using decision-analytic modeling. For instance, Hepatitis C Disease Burden Simulation (HEP-SIM) has projected the changing HCV prevalence and associated disease burden in the United States from 2001 to 2050 [22, 23]. The HEP-SIM model closely replicated the HCV prevalence as estimated by the National Health and Nutrition Examination Survey (NHANES). The model predicted that the number of viremic patients will decrease over time—from 2.5 million people in 2010 to below 1.0 million by 2020 (Fig. 3.3). At the same time, the number of individuals living with a sustained virologic response (SVR), a surrogate for HCV cure, is expected to increase from 0.8 million to 1.6 million by 2020. This study showed that despite the expected decrease of HCV-associated outcomes with implementation of the DAA therapy, HCC incidence would continue to increase until 2020. However, the cumulative incidences of HCC and LRD from 2015 to 2050 would be reduced substantially. It was estimated that

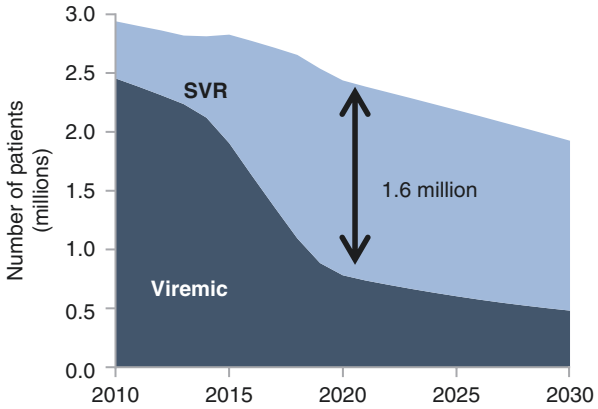

Fig. 3.3 Predicted trend of HCV-related patients. SVR, sustained virologic response

the cumulative incidence of HCC when treated with DAAs, pre-DAA therapies, and no treatment from 2015 to 2050 were 157,000, 305,000, and 415,000 respectively, while the corresponding liver-related deaths were 320,000, 587,000, and 776,000, respectively. Moreover, the study showed that increasing the annual treatment rate to 280,000 from 2015 onward would prevent 5400 cases of HCC and 9700 deaths.

Another modeling study projected trends in NASH and related disease burden from 2015 to 2030 [29]. The study estimated that the number of NASH cases would increase by 63% from year 2015 to year 2030. The prevalence of NASH-associated HCC would increase by 146% from 10,100 to 24,900 from 2015 to 2030. The incidence rate of NASH-HCC is also expected to increase by 137% from 5200 in 2015 to 12,200 in 2030. With potential availability of NASH treatments in the near future, NASH-associated HCC could be lower than what has been projected by the above studies.

While ALD accounts for 30% of all the HCC cases worldwide [10], the future burden of ALD-associated HCC has not been well studied [10, 30]. The Global Burden of Disease Study estimated the proportion of ALD-HCC among all-cause HCC cases across different geological regions as shown in Table 3.1. HCC patients with ALD are more prone to adverse disease prognosis and less frequent access to curative therapies [9, 31–36]. The Global Burden of Disease Study concluded that the contribution of alcoholic is expected to increase further due to improved preventive and treatment measures of nonalcoholic HCC etiologies, such as HBV vaccination, HCV treatment with DAA therapies, and potential stabilization of obesity in the United States, Europe, and China [12, 30, 37].

Knowledge Gaps in HCC Surveillance

Though HCC surveillance can detect early-stage treatable cancers, its use remains controversial and highly variable in practice [7]. Many professional societies recommend regular HCC surveillance in high-risk patients (e.g., having cirrhosis)

using liver ultrasound and/or AFP measurements to increase the likelihood of detecting early-stage treatable cancer [38–40]. However, the US Preventive Services Task Force (USPSTF) does not endorse routine HCC surveillance.

Several gaps remain in the comparative effectiveness data that inform surveillance policies [41]. First, conclusive evidence from randomized controlled trials (RCTs) showing that HCC surveillance reduces mortality is lacking. To date, only two clinical trials have evaluated the effectiveness of HCC screening—one found that surveillance was effective in reducing all-cause mortality, but the second did not find any benefits [42, 43]. Notably, these studies included only HBV patients from China; no trial has evaluated screening in patients with other etiologies of HCC, which cause 85–90% of all HCC cases in the United States. Any future trial evaluating a no-screening scenario may be deemed unethical because clinical practice guidelines are already in place [44]. Furthermore, the vast majority of patients are not willing to participate in trials that have a no-screening arm [45]. Many observational studies have shown that HCC surveillance increases survival [46]; however, these studies are prone to lead- and length-time bias [47, 48], as has been observed in other cancer screening programs [49–51].

Second, the current screening guidelines fail to capture many at-risk individual [52]. Though current guidelines primarily recommend surveillance in cirrhotic patients, 20–50% of patients presenting with HCC have previously undiagnosed cirrhosis [53]. Furthermore, nearly 50% of all cases of HCC originate in noncirrhotic livers, and HCV patients without cirrhosis are also at risk of developing HCC [54–57]. Many of these patients would not enter into a surveillance program if the presence of cirrhosis alone were used to define a target population. Therefore, data on the risk and benefit of regular surveillance in this cohort are needed.

Third, existing guidelines do not tailor surveillance according to an individual's risk and instead follow a "one-size-fits-all" paradigm, making them inefficient or ineffective for many individuals [58]. For a successful effectiveness program, it is important to distinguish patients in whom aggressive surveillance in needed from those who require less frequent surveillance, if at all. Despite the heterogeneous nature of HCC, published studies have not evaluated individualized surveillance based on patients' risk factors including age, liver disease, comorbidity, and access to liver transplant. For example, current guidelines do not specify when to start or stop surveillance in most patients, which makes it difficult to define the populations for which surveillance could be cost-effective. Furthermore, recent exciting advances in molecular biomarkers that can aid in early HCC detection have not been incorporated in surveillance guidelines [59].

Fourth, current surveillance practices are based on the cost-effectiveness studies that have not considered recent changes in liver transplant practice and advances in the treatment of HCC [38, 60–63] and therefore could have underestimated the value of surveillance.

Finally, there is a need to balance the benefits of regular surveillance with harms. Potential harms of HCC screening include serious complications from liver biopsy, which occur in about 1% of patients [64], and, rarely, death from liver biopsy (0.009–0.12% patients) [65]. Other harms include complications from liver resection and from other tests and treatments offered because of findings from ultrasonography

[66]. Surveillance could also result in the overdiagnosis of HCC in patients with comorbidities, especially in older age, who are not eligible for curative treatments such as liver transplantation.

Significance of Mathematical Modeling for Surveillance of HCC

RCTs on HCC surveillance would likely be prohibitively expensive, time-consuming, and considered unethical by many. To sufficiently address the evidence gaps, a large RCT will need to incorporate and compare a prohibitively large number of arms [67], which is not realistically achievable. Under such situation, mathematical modeling can capture many of the complex intricacies to healthcare delivery in the real world, incorporate risks and benefits, and predict the long-term outcomes of different strategies that can guide decision-makers in establishing evidence-based guidelines [67, 68]. Results of such analyses have been used by the USPSTF and the Centers for Medicare and Medicaid Services (CMS) for determining screening recommendations for breast [69], colorectal [70, 71], cervical [72], and lung cancers [73].

To develop an effective and cost-effective surveillance for HCC, the first step is to determine which patients will (or will not) benefit from routine surveillance. Populations at risk of developing HCC are highly heterogeneous because of multiple possible etiologies, liver disease stage, comorbidities, and access to treatment. Mathematical modeling can consider such complex dynamics and provide insights to determine the frequency of surveillance personalized to individual's risk factors based on clinical and/or molecular indices [74].

Cost-Effectiveness of HCC Surveillance by Etiology

The risk of developing HCC varies with the underlying etiology—5-year cumulative risk is 17% in patients with HCV cirrhosis, 10% with HBV cirrhosis, and 2–8% with alcoholic cirrhosis [26, 75]. Therefore, a surveillance policy tailored to the underlying etiology could be more effective and cost-effective than a single policy across all etiologies. Another gap in the current surveillance recommendations is that they exclude noncirrhotic patients (except for HBV). However, up to 54% of all cases of HCC originate in noncirrhotic livers according to various etiologies [56, 57]. Finally, many HCV patients have multiple comorbidities; therefore, a surveillance program needs to weigh the harms and benefits in this population.

Hepatitis C Patients After Viral Cure

A recent retrospective cohort study using data from the VA HCV Clinical Case Registry showed that the annual risk of HCC remained considerably high among patients with cirrhosis (1.39%/year) and those cured after age 64 (0.95%/year) [54]. Patients with diabetes (adjusted HR = 1.88) or HCV genotype 3 infection (adjusted HR = 1.62) were also more likely to develop HCC. In an ongoing work, we further predicted the number of HCV patients who will develop HCC after a successful antiviral treatment. These data highlight the importance (and urgency) of an effective surveillance policy in HCV patients after a successful treatment. However, there are no data to guide optimal surveillance policies—including when to stop surveillance [54, 76].

Nonalcoholic Fatty Liver Disease

A systematic review of 61 studies showed that although patients with NAFLD or NASH without cirrhosis had a low risk for HCC [77], the risk was considerably higher in NASH cirrhosis (cumulative incidence ranging from 2.4% over 7 years to 12.8% over 3 years). Because NASH patients are different from patients with viral hepatitis, we should not generalize HCC surveillance policies from other etiologies to this growing group. This work emphasizes the need for data on the benefits and harms of HCC surveillance in this population.

Patients with NAFLD or NASH who have cirrhosis have a high risk of developing HCC [77]. In addition, a recent study found that 13% of patients with HCC did not have cirrhosis [78]. Patients with NASH also have the highest risk for unrecognized liver disease, which makes timely diagnosis of HCC even more difficult [77]. Risk of liver-related death is higher in NASH patients, but death from cardiovascular disease is the most common cause [79–82]. The association with cardiovascular disease suggests that the cost-effectiveness of HCC surveillance in patients with NAFLD or NASH could be different from that in other etiologies [83].

Alcoholic Liver Disease

Risk of HCC in ALD is lower than that in other major etiologies [26, 75]. Though the current guidelines recommend surveillance in patients with ALD-associated cirrhosis, a recent study questioned the value of HCC surveillance in this population [26, 75]. There is a need to evaluate the effectiveness and cost-effectiveness of

surveillance in this group. Because HCC can also arise without established cirrhosis in 14–19% ALD patients [84], there is also a need to evaluate the value of surveillance in both cirrhotic and noncirrhotic ALD patients.

Heavy Alcohol Consumption with HBV/HCV

Heavy alcohol consumption significantly increases the risk of HCC in HCV and HBV patients [85]. The risk for HCC increases five times with a daily alcohol consumption of 80 g; and a combination of both HCV and alcohol leads to a 100-fold risk for HCC development [86]. Similarly, the 10-year cumulative HCC incidence is significantly higher for cirrhotic patients with HBV infection and alcoholism than for those with HBV or alcoholism alone (52.8%, 39.8%, and 25.6%, respectively) [87].

Conclusions

HCC is responsible for 80–90% of primary liver cancer cases and is the third most common cause of cancer deaths worldwide. In the United States, HCC is the fastest growing cause of cancer deaths. HCC incidence has increased threefold between 1975 and 2009. HCC etiology has changed in the last decade and is further projected to change. HCV-associated HCC is projected to decrease substantially because of the availability of new antivirals for HCV; however, NAFLD- and ALD-associated HCC is likely to increase in the near future.

Routine surveillance plays an important role in early detection of HCC, but the adherence to surveillance remains low. In order to increase the value of surveillance programs, surveillance should be tailored to underlying etiology and other risk factors. Current screening guidelines are based on cost-effectiveness data that are old, and there is a need to evaluate the new threshold to HCC incidence above which routine HCC surveillance can be deemed cost-effective.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90. https://doi.org/10.3322/caac.20107.
2. World Cancer Report 2014. World Health Organization. 2014. Chapter 1.1. ISBN 9283204298.
3. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264–73 e1. https://doi.org/10.1053/j.gastro.2011.12.061.
4. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009;27(9):1485–91.

5. Ryerson AB, Eheman CR, Altekruse SF, Ward JW, Jemal A, Sherman RL, et al. Annual Report to the Nation on the Status of Cancer, 1975–2012, featuring the increasing incidence of liver cancer. Cancer. 2016. https://doi.org/10.1002/cncr.29936.
6. Singal AG, Tiro JA, Gupta S. Improving hepatocellular carcinoma screening: applying lessons from colorectal cancer screening. Clin Gastroenterol Hepatol. 2013;11(5):472–7. https://doi.org/10.1016/j.cgh.2012.11.010.
7. van Meer S, de Man RA, Siersema PD, van Erpecum KJ. Surveillance for hepatocellular carcinoma in chronic liver disease: evidence and controversies. World J Gastroenterol. 2013;19(40):6744–56. https://doi.org/10.3748/wjg.v19.i40.6744.
8. Davila JA, Henderson L, Kramer JR, Kanwal F, Richardson PA, Duan Z, et al. Utilization of surveillance for hepatocellular carcinoma among hepatitis C virus-infected veterans in the United States. Ann Intern Med. 2011;154(2):85–93. https://doi.org/10.7326/0003-4819-154-2-201101180-00006.
9. Davila JA, Morgan RO, Richardson PA, Du XL, McGlynn KA, El-Serag HB. Use of surveillance for hepatocellular carcinoma among patients with cirrhosis in the United States. Hepatology (Baltimore, Md). 2010;52(1):132–41. https://doi.org/10.1002/hep.23615.
10. Global Burden of Disease Liver Cancer Collaboration, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. JAMA Oncol. 2017;3(12):1683–91. https://doi.org/10.1001/jamaoncol.2017.3055.
11. Chhatwal J, Chen Q, Bethea E, Ladd M, Mueller P, Hutin Y, et al. Hep C Calculator: an online tool for cost-effectiveness analysis of DAAs. Lancet Gastroenterol Hepatol. 2018;3(12):819. https://doi.org/10.1016/S2468-1253(18)30281-4.
12. Goh GB, Chang PE, Tan CK. Changing epidemiology of hepatocellular carcinoma in Asia. Best Pract Res Clin Gastroenterol. 2015;29(6):919–28. https://doi.org/10.1016/j.bpg.2015.09.007.
13. Lee CM, Lu SN, Changchien CS, Yeh CT, Hsu TT, Tang JH, et al. Age, gender, and local geographic variations of viral etiology of hepatocellular carcinoma in a hyperendemic area for hepatitis B virus infection. Cancer. 1999;86(7):1143–50.
14. Liang X, Bi S, Yang W, Wang L, Cui G, Cui F, et al. Epidemiological serosurvey of hepatitis B in China—declining HBV prevalence due to hepatitis B vaccination. Vaccine. 2009;27(47):6550–7.
15. Ni YH, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, et al. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. Ann Intern Med. 2001;135(9):796–800.
16. Ang LW, Cutter J, James L, Goh KT. Seroepidemiology of hepatitis B virus infection among adults in Singapore: a 12-year review. Vaccine. 2013;32(1):103–10. https://doi.org/10.1016/j.vaccine.2013.10.057.
17. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351(15):1521–31. https://doi.org/10.1056/NEJMoa033364.
18. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology. 1999;29(3):971–5. https://doi.org/10.1002/hep.510290312.
19. Farrell GC, Wong VW-S, Chitturi S. NAFLD in Asia—as common and important as in the West. Nat Rev Gastroenterol Hepatol. 2013;10(5):307.
20. White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of hepatocellular carcinoma in all 50 United States, from 2000 through 2012. Gastroenterology. 2017;152(4):812–20. e5.
21. Singal AG, Murphy CC. Hepatocellular carcinoma: a roadmap to reduce incidence and future burden. J Natl Cancer Inst. 2018. https://doi.org/10.1093/jnci/djy184.
22. Kabiri M, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J. The changing burden of hepatitis C in the United States: model-based predictions. Ann Intern Med. 2014;161(3):170–80. https://doi.org/10.7326/M14-0095.

23. Chhatwal J, Wang X, Ayer T, Kabiri M, Chung RT, Hur C, et al. Hepatitis C disease burden in the United States in the era of oral direct-acting antivirals. Hepatology (Baltimore, Md). 2016;64(5):1442–50.
24. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. Hepatology. 2014;59(6):2188–95. https://doi.org/10.1002/hep.26986.
25. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol. 2012;56(6):1384–91. https://doi.org/10.1016/j.jhep.2011.10.027.
26. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatology (Baltimore, Md). 2014;60(5):1767–75. https://doi.org/10.1002/hep.27222.
27. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999–2016: observational study. BMJ. 2018;362:k2817. https://doi.org/10.1136/bmj.k2817.
28. Razavi H, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, et al. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. J Viral Hepat. 2014;21(Suppl 1):34–59. https://doi.org/10.1111/jvh.12248.
29. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology. 2018;67(1):123–33. https://doi.org/10.1002/hep.29466.
30. Ganne-Carrie N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. J Hepatol. 2019;70(2):284–93. https://doi.org/10.1016/j.jhep.2018.10.008.
31. Edenvik P, Davidsdottir L, Oksanen A, Isaksson B, Hultcrantz R, Stal P. Application of hepatocellular carcinoma surveillance in a European setting. What can we learn from clinical practice? Liver Int. 2015;35(7):1862–71. https://doi.org/10.1111/liv.12764.
32. Schutte K, Bornschein J, Kahl S, Seidensticker R, Arend J, Ricke J, et al. Delayed diagnosis of HCC with chronic alcoholic liver disease. Liver Cancer. 2012;1(3–4):257–66. https://doi.org/10.1159/000343840.
33. Bucci L, Garuti F, Camelli V, Lenzi B, Farinati F, Giannini E, et al. Comparison between alcohol-and hepatitis C virus-related hepatocellular carcinoma: clinical presentation, treatment and outcome. Aliment Pharmacol Ther. 2016;43(3):385–99.
34. Singal AG, Yopp AC, Gupta S, Skinner CS, Halm EA, Okolo E, et al. Failure rates in the hepatocellular carcinoma surveillance process. Cancer Prev Res (Phila). 2012;5(9):1124–30. https://doi.org/10.1158/1940-6207.CAPR-12-0046.
35. Trevisani F, Magini G, Santi V, Morselli-Labate AM, Cantarini MC, Di Nolfo MA, et al. Impact of etiology of cirrhosis on the survival of patients diagnosed with hepatocellular carcinoma during surveillance. Am J Gastroenterol. 2007;102(5):1022–31. https://doi.org/10.1111/j.1572-0241.2007.01100.x.
36. Costentin CE, Mourad A, Lahmek P, Causse X, Pariente A, Hagege H, et al. Hepatocellular carcinoma is diagnosed at a later stage in alcoholic patients: results of a prospective, nationwide study. Cancer. 2018;124(9):1964–72. https://doi.org/10.1002/cncr.31215.
37. Guirguis J, Chhatwal J, Dasarathy J, Rivas J, McMichael D, Nagy LE, et al. Clinical impact of alcohol-related cirrhosis in the next decade: estimates based on current epidemiological trends in the United States. Alcohol Clin Exp Res. 2015;39(11):2085–94. https://doi.org/10.1111/acer.12887.
38. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology (Baltimore, Md). 2011;53(3):1020–2. https://doi.org/10.1002/hep.24199.
39. Llovet JM, Lencioni R, Di Bisceglie AM, Galle PR, Dufour JF, Greten TF, Raymond E, Roskams T, De Baere T, Ducreux M, Mazzaferro V, Bernardi M, Bruix J, Colombo M, Zhu A. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43.
40. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010;4(2):439–74. https://doi.org/10.1007/s12072-010-9165-7.

41. Kansagara D, Papak J, Pasha AS, O'Neil M, Freeman M, Relevo R, et al. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. Ann Intern Med. 2014;161(4):261–9. https://doi.org/10.7326/M14-0558.
42. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417–22. https://doi.org/10.1007/s00432-004-0552-0.
43. Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, et al. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. J Med Screen. 2003;10(4):204–9. https://doi.org/10.1258/096914103771773320.
44. Poustchi H, Farrell GC, Strasser SI, Lee AU, McCaughan GW, George J. Feasibility of conducting a randomized control trial for liver cancer screening: is a randomized controlled trial for liver cancer screening feasible or still needed? Hepatology (Baltimore, Md). 2011;54(6):1998–2004. https://doi.org/10.1002/hep.24581.
45. Kanwal F, El-Serag HB, Ross D. Surveillance for hepatocellular carcinoma: can we focus on the mission? Clin Gastroenterol Hepatol. 2015;13(4):805–7. https://doi.org/10.1016/j.cgh.2014.12.016.
46. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. https://doi.org/10.1371/journal.pmed.1001624.
47. Duffy SW, Nagtegaal ID, Wallis M, Cafferty FH, Houssami N, Warwick J, et al. Correcting for lead time and length bias in estimating the effect of screen detection on cancer survival. Am J Epidemiol. 2008;168(1):98–104. https://doi.org/10.1093/aje/kwn120.
48. Cucchetti A, Garuti F, Pinna AD, Trevisani F. Length time bias in surveillance for hepatocellular carcinoma diagnosis and how to avoid it. Hepatol Res. 2016. https://doi.org/10.1111/hepr.12672.
49. Bleyer A, Welch HG. Effect of three decades of screening mammography on breast-cancer incidence. N Engl J Med. 2012;367(21):1998–2005. https://doi.org/10.1056/NEJMoa1206809.
50. Etzioni R, Penson DF, Legler JM, di Tommaso D, Boer R, Gann PH, et al. Overdiagnosis due to prostate-specific antigen screening: lessons from U.S. prostate cancer incidence trends. J Natl Cancer Inst. 2002;94(13):981–90.
51. Wang JH, Chang KC, Kee KM, Chen PF, Yen YH, Tseng PL, et al. Hepatocellular carcinoma surveillance at 4- vs. 12-month intervals for patients with chronic viral hepatitis: a randomized study in community. Am J Gastroenterol. 2013;108(3):416–24. https://doi.org/10.1038/ajg.2012.445.
52. McMahon B, Block J, Block T, Cohen C, Evans AA, Hosangadi A, et al. Hepatitis-associated liver cancer: gaps and opportunities to improve care. J Natl Cancer Inst. 2016;108(4). https://doi.org/10.1093/jnci/djv359.
53. Zaman SN, Johnson PJ, Williams R. Silent cirrhosis in patients with hepatocellular carcinoma. Implications for screening in high-incidence and low-incidence areas. Cancer. 1990;65(7):1607–10.
54. El-Serag HB, Kanwal F, Richardson P, Kramer J. Risk of hepatocellular carcinoma after sustained virologic response in veterans with HCV-infection. Hepatology (Baltimore, Md). 2016;64:130–7. https://doi.org/10.1002/hep.28535.
55. Lok AS, Seeff LB, Morgan TR, Di Bisceglie AM, Sterling RK, Curto TM, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009;136(1):138–48.
56. Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinico-histopathologic study of 804 North American patients. Am J Clin Pathol. 1996;105(1):65–75.
57. Brancatelli G, Federle MP, Grazioli L, Carr BI. Hepatocellular carcinoma in noncirrhotic liver: CT, clinical, and pathologic findings in 39 U.S. residents. Radiology. 2002;222(1):89–94. https://doi.org/10.1148/radiol.2221010767.
58. Goossens N, Bian CB, Hoshida Y. Tailored algorithms for hepatocellular carcinoma surveillance: is one-size-fits-all strategy outdated? Curr Hepatol Rep. 2017;16(1):64–71. https://doi.org/10.1007/s11901-017-0336-z.

59. Abu Dayyeh BK, Yang M, Fuchs BC, Karl DL, Yamada S, Sninsky JJ, et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology. 2011;141(1):141–9. https://doi.org/10.1053/j.gastro.2011.03.045.
60. Wang Y, Luo Q, Li Y, Deng S, Wei S, Li X. Radiofrequency ablation versus hepatic resection for small hepatocellular carcinomas: a meta-analysis of randomized and nonrandomized controlled trials. PLoS One. 2014;9(1):e84484. https://doi.org/10.1371/journal.pone.0084484.
61. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90. https://doi.org/10.1056/NEJMoa0708857.
62. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;389(10064):56–66. https://doi.org/10.1016/S0140-6736(16)32453-9.
63. Kudo M. Regorafenib as second-line systemic therapy may change the treatment strategy and management paradigm for hepatocellular carcinoma. Liver Cancer. 2016;5(4):235–44. https://doi.org/10.1159/000449335.
64. Seeff LB, Everson GT, Morgan TR, Curto TM, Lee WM, Ghany MG, et al. Complication rate of percutaneous liver biopsies among persons with advanced chronic liver disease in the HALT-C trial. Clin Gastroenterol Hepatol. 2010;8(10):877–83. https://doi.org/10.1016/j.cgh.2010.03.025.
65. Tobkes AI, Nord HJ. Liver biopsy: review of methodology and complications. Dig Dis (Basel, Switzerland). 1995;13(5):267–74.
66. Lederle FA, Pocha C. Screening for liver cancer: the rush to judgment. Ann Intern Med. 2012;156(5):387–9. https://doi.org/10.7326/0003-4819-156-5-201203060-00012.
67. Sherman M. Whither hepatocellular carcinoma screening? Hepatology (Baltimore, Md). 2012;56(6):2412–4. https://doi.org/10.1002/hep.26138.
68. Chirovsky D, Lich KH, Barritt AS. Screening for hepatocellular carcinoma in chronic liver disease. Ann Intern Med. 2015;162(3):238–9. https://doi.org/10.7326/l15-5049.
69. Mandelblatt JS, Cronin KA, Bailey S, Berry DA, de Koning HJ, Draisma G, et al. Effects of mammography screening under different screening schedules: model estimates of potential benefits and harms. Ann Intern Med. 2009;151(10):738–47. https://doi.org/10.7326/0003-4819-151-10-200911170-00010. 151/10/738 [pii].
70. Knudsen AB, Lansdorp-Vogelaar I, Rutter CM, Savarino JE, van Ballegooijen M, Kuntz KM, et al. Cost-effectiveness of computed tomographic colonography screening for colorectal cancer in the medicare population. J Natl Cancer Inst. 2010;102(16):1238–52. https://doi.org/10.1093/jnci/djq242. djq242 [pii].
71. Lansdorp-Vogelaar I, Kuntz KM, Knudsen AB, Wilschut JA, Zauber AG, van Ballegooijen M. Stool DNA testing to screen for colorectal cancer in the Medicare population: a cost-effectiveness analysis. Ann Intern Med. 2010;153(6):368–77. https://doi.org/10.7326/0003-4819-153-6-201009210-00004.
72. Kulasingam SL, Havrilesky LJ, Ghebre R, Myers ER. Screening for cervical cancer: a modeling study for the US Preventive Services Task Force. J Low Genit Tract Dis. 2013;17(2):193–202. https://doi.org/10.1097/LGT.0b013e3182616241.
73. de Koning HJ, Meza R, Plevritis SK, Ten Haaf K, Munshi VN, Jeon J, et al. Benefits and harms of computed tomography lung cancer screening strategies: a comparative modeling study for the U.S. Preventive Services Task Force. Ann Intern Med. 2013. https://doi.org/10.7326/M13-2316. 1809424 [pii].
74. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, Besa C, et al. Cost-effectiveness of risk score-stratified hepatocellular carcinoma screening in patients with cirrhosis. Clin Transl Gastroenterol. 2017;8(6):e101. https://doi.org/10.1038/ctg.2017.26.
75. Jepsen P, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Risk for hepatocellular carcinoma in patients with alcoholic cirrhosis: a Danish nationwide cohort study. Ann Intern Med. 2012;156(12):841–7. https://doi.org/10.7326/0003-4819-156-12-201206190-00004.

76. D'Ambrosio R, Colombo M. Should surveillance for liver cancer be modified in hepatitis c patients after treatment-related cirrhosis regression? Liver Int. 2016. https://doi.org/10.1111/liv.13106.
77. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. Clin Gastroenterol Hepatol. 2012;10(12):1342–59.e2. https://doi.org/10.1016/j.cgh.2012.10.001.
78. Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, et al. Hepatocellular carcinoma in the absence of cirrhosis in United States veterans is associated with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2016;14(1):124–31 e1. https://doi.org/10.1016/j.cgh.2015.07.019.
79. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology (Baltimore, Md). 2010;51(2):595–602. https://doi.org/10.1002/hep.23314.
80. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol. 2008;49(4):608–12. https://doi.org/10.1016/j.jhep.2008.06.018.
81. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol. 2009;7(2):234–8. https://doi.org/10.1016/j.cgh.2008.11.005.
82. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology (Baltimore, Md). 2006;44(4):865–73. https://doi.org/10.1002/hep.21327.
83. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med. 2010;363(14):1341–50. https://doi.org/10.1056/NEJMra0912063.
84. Yeh MM, Daniel HD, Torbenson M. Hepatitis C-associated hepatocellular carcinomas in non-cirrhotic livers. Mod Pathol. 2010;23(2):276–83. https://doi.org/10.1038/modpathol.2009.174.
85. Vandenbulcke H, Moreno C, Colle I, Knebel JF, Francque S, Serste T, et al. Alcohol intake increases the risk of HCC in hepatitis C virus-related compensated cirrhosis: a prospective study. J Hepatol. 2016;65(3):543–51. https://doi.org/10.1016/j.jhep.2016.04.031.
86. Mueller S, Millonig G, Seitz HK. Alcoholic liver disease and hepatitis C: a frequently underestimated combination. World J Gastroenterol: WJG. 2009;15(28):3462–71. https://doi.org/10.3748/wjg.15.3462.
87. Lin C-W, Lin C-C, Mo L-R, Chang C-Y, Perng D-S, Hsu C-C, et al. Heavy alcohol consumption increases the incidence of hepatocellular carcinoma in hepatitis B virus-related cirrhosis. J Hepatol. 2013;58(4):730–5. https://doi.org/10.1016/j.jhep.2012.11.045.

Part II
Diagnosis and Prognostication

Chapter 4
Radiological Diagnosis and Characterization of HCC

Naik Vietti Violi, Sara Lewis, Stefanie Hectors, Daniela Said, and Bachir Taouli

Abbreviations

AASLD	American Association for the Study of Liver Diseases
ADC	Apparent diffusion coefficient
AFP	Alpha-fetoprotein
BCLC	Barcelona clinic liver cancer
CE-CT	Contrast-enhanced computed tomography
CEUS	Contrast-enhanced ultrasound
CT	Computed tomography
DCE	Dynamic contracted enhanced

The original version of this chapter was revised. The correction to this chapter can be found at https://doi.org/10.1007/978-3-030-21540-8_17

N. Vietti Violi
Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Lausanne University hospital and University of Lausanne, Lausanne, Switzerland
e-mail: naik.viettivioli@mssm.edu

S. Lewis · S. Hectors · B. Taouli (✉)
Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
e-mail: sara.lewis@mountsinai.org; stefanie.hectors@mountsinai.org; bachir.taouli@mountsinai.org

D. Said
Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Radiology, Universidad de los Andes, Santiago, Chile
e-mail: daniela.said@mssm.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_4

DECT	Dual energy CT
DN	Dysplastic nodule
DWI	Diffusion-weighted imaging
EASL	European Association for the Study of Liver
ECCM	Extracellular contrast media
HBP	Hepatobiliary phase
HCC	Hepatocellular carcinoma
HGDN	High-grade dysplastic nodule
IVIM	Intravoxel incoherent motion
LI-RADS	Liver Imaging Reporting and Data System
MRI	Magnetic resonance imaging
NASH	Nonalcoholic steatohepatitis
OPTN	Organ Procurement and Transplantation Network
PET	Positron emission tomography
TACE	Transarterial chemoembolization
US	Ultrasound

Introduction

Over the past 20 years, noninvasive imaging has played a central role in the diagnosis of hepatocellular carcinoma (HCC). Biopsies in patients with chronic liver disease are not routinely performed for diagnosis because HCC diagnosis can be made using imaging with high specificity and reasonable sensitivity (depending on tumor size), according to the current practice guidelines [1, 2]. In addition, patients with chronic liver disease are at risk of post-procedure complications [3]. There is also a risk of neoplastic seeding on the biopsy tract [4], emphasizing the need interest of noninvasive diagnosis in this population. Radiologists are involved in most important aspects of HCC management including screening, surveillance, diagnosis, staging, and assessment of posttreatment response [5, 6]. Imaging methods can also be used for guidance for percutaneous biopsy and for HCC treatment using locoregional therapies (LRT).

Abdominal ultrasound (US) is the imaging modality of choice for HCC screening, as recommended by the American Association for the Study of Liver Diseases (AASLD). The role of serum alpha-fetoprotein (AFP) for screening is limited, and serum AFP is optional in the latest AASLD practice guidelines [1, 7]. Contrast-enhanced US (CEUS) provides dynamic assessment of tumor contrast enhancement in addition to gray-scale US evaluation. However, its use is still limited in the United States. Contrast-enhanced multiphasic computed tomography (CE-CT) and magnetic resonance imaging (MRI) are the mostly used modalities for characterizing liver lesions in cirrhotic patients, at risk for HCC.

HCC diagnosis can be confidently made with high specificity based on the imaging characteristics on CT or MRI, without pathologic confirmation needed in typical cases. According to the AASLD and the European Association for the Study of Liver (EASL), HCC diagnosis is indeed based either on pathological examination or noninvasive imaging criteria [4, 8]. These criteria are based on three findings/

conditions that must be present: (1) patient with cirrhosis, (2) presenting a liver nodule >10 mm, and (3) with characteristic vascular features compared to the adjacent liver parenchyma in a cross-sectional imaging study using intravenous iodine or gadolinium-based contrast agents: hypervascular lesion in the arterial phase (wash-in) and washout in the portal venous (60–70 s postinjection) or delayed venous (180 s postinjection) phases. In the absence of one of these criteria, radiological diagnosis of HCC cannot be made, and further investigations (other imaging tools/biopsy/follow-up) are required. Multiple national and international practice guidelines have also been developed with some local differences but with a consensus on the concept of noninvasive diagnosis: the Asian Pacific Association for the Study of the Liver (APASL) [9], the Japan Society of Hepatology [10], and the Italian Association for the Study of the Liver [11]. The diagnostic pathway from the liver lesion detection to the diagnosis has also been standardized [1, 7].

Based on HCC radiologic diagnosis criteria, the Liver Imaging Reporting and Data System (LI-RADS) was introduced in 2011 and endorsed by the American College of Radiology (ACR) [12]. The goal is to provide a standardized interpretation and reporting system of CT and MRI performed in patients at risk of HCC. The LI-RADS classified imaging observations from definitely benign (LR-1) to definitely HCC (LR-5) using HCC diagnosis main features (arterial hyperenhancement, lesion size, washout, capsule appearance, and threshold growth) and ancillary features favoring either malignancy or benignity (see below). Additional classifications have been added for treated lesions (LR-T), lesions suspicious for malignancy but not HCC (LR-M), and HCC with macrovascular invasion (LR-TIV: tumor in vein) due to important implications for patient management [13]. In addition, the LI-RADS provides guidelines for radiologists performing, interpreting, and reporting the radiologic images and suggests patient management. This classification has shown to be accurate even with lesion size <2 cm with a high specificity for diagnosing HCC (96.4% for LI-RADS category 4 and 5) and sensitivity of 65.4% [14]. The LI-RADS is now extending worldwide and has recently merged with the AASLD diagnostic criteria [15].

Imaging Modalities

Ultrasound and Contrast-Enhanced Ultrasound

US is the most frequent first imaging modality used in abdomen due to its low cost, wide availability, and noninvasiveness. It is the modality of choice for HCC screening and surveillance because of its advantages and its high specificity that reaches over 90% for detecting HCC [16]. However, US sensitivity is limited in the background of cirrhosis and obesity and for detecting small HCC <20 mm, described as low as 27.9% in UNOS T1/T2 HCC in a recent prospective Korean study [17]. US is also operator-dependent. Due in part to these challenges, US sensitivity for early-stage HCC, potentially curative disease, is low. Nevertheless, currently no

alternative to US is appropriate for screening because of higher cost, radiation exposure (CT), and long exam times for CE-MRI (at least 30 min). As a result, current practice guidelines do not advocate multiphasic CE-CT or CE-MRI for HCC surveillance.

On US, HCC with a size <30 mm will typically appear hypoechoic. Lesion heterogeneity could be due to either fat, hemorrhage, or necrosis. A hyperechoic focus within a hypoechoic mass may be suggestive of an HCC developed in a dysplastic nodule (DN) [18]. Lesions with a size ≥30 mm may exert mass effect on adjacent structures and invade the portal vein and its branches. Larger lesions tend to be more heterogeneous and poorly defined or may present with hypoechoic halo [19].

CEUS is based on a blood pool agent (sulfur hexafluoride, Lumason, Bracco Imaging, and perflutren protein-type A microspheres, Optison, GE Healthcare, both approved for liver imaging in the United States) that remains confined to the vascular space, without interstitial distribution like CT and MR contrasts. Compared to gray-scale US, CEUS assesses dynamic evaluation of lesion contrast enhancement with real-time evaluation of all different phases with identification of the wash-in and washout features mirroring multiphasic CT or MRI. CEUS has the advantage over CT and MRI as it is less costly and it allows a dynamic contrast evaluation. It has shown excellent sensitivity for detection of hypervascular lesions [20–22]. CEUS use is still very limited in the United States, due to recent FDA approval of the contrast agents. In addition, CEUS has the same limitations as conventional US, such as operator dependence, limited sensitivity in obese and cirrhotic patients and for small lesions, and limited detection of deep liver lesions [23].

Contrast-Enhanced CT

Contrast-enhanced CT (CE-CT) is a valuable technique for HCC diagnosis. As recommended by UNOS guidelines [2], multiphasic CT for HCC diagnosis should include four phases: (1) non-contrast phase in order to detect hyperdense structures (such as hemorrhage or changes related to locoregional therapy); (2) late arterial phase, corresponding to the peak of tumor enhancement; (3) portal venous phase (60–70 s postinjection) corresponding to the peak of portal venous and parenchymal enhancement within the liver and the most adequate for venous evaluation; and (4) the delayed venous phase (180 s postinjection) which increases detection of tumor capsule [24] (Fig. 4.1).

While CT sensitivity for nodular HCC ≥2 cm can be as high as 90%, it falls significantly for lesions with a size between 1 cm and 2 cm (40–44%) or <1 cm (10–33%) [25]. CT has the advantage over MRI as being widely available, rapid, and robust, and images do not need an advanced expertise to be interpreted. The main disadvantages of CT are the radiation exposure, the lower contrast resolution compared to MRI, and lower sensitivity for small lesions as indicated above [25].

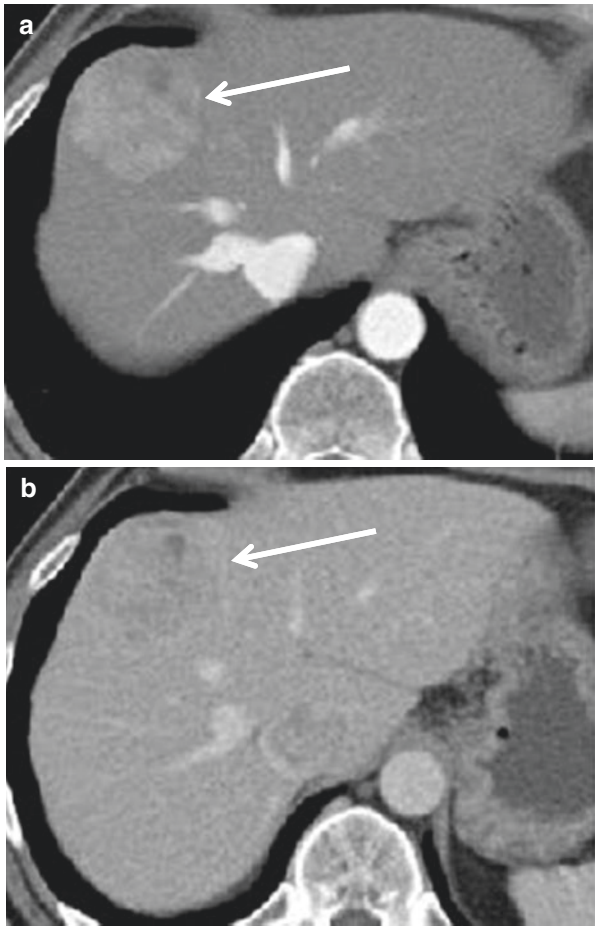

Fig. 4.1 A 72-year-old male patient with chronic hepatitic C virus cirrhosis and HCC. (**a**) Axial contrast-enhanced CT acquired during the arterial phase demonstrates a large (4.5 cm) mass in segment VIII with arterial hyperenhancement (wash-in, arrow). (**b**) Axial contrast-enhanced CT during the portal venous phase demonstrates lesion washout with enhancing capsule (arrow)

MRI

Liver MRI can be performed using two different gadolinium-based contrast agents (GBCA): extracellular contrast media (ECCM), which have the same behavior as iodinated contrast agent used for CT, and liver-specific contrast agents [gadobenate dimeglumine, MultiHance, Bracco Diagnostics, and gadoxetic acid (Gd-EOB-DTPA), Eovist/Primovist, Bayer Healthcare]. Liver-specific agents can be used initially as dynamic agents and eventually penetrate the hepatocytes (small portion 3–5% for gadobenate dimeglumine and up to 50% for gadoxetic acid) and are excreted into the biliary system. Although the use of liver-specific agents for HCC diagnosis is increasing, ECCM remain the reference contrast agents for this purpose.

MRI with ECCM

Similar to CT, dedicated liver MRI must include multiple vascular phases acquired at pre-contrast, arterial (one or multiple arterial phases), portal venous (60 s postinjection), and delayed venous (180 s postinjection) phases with the same goal as CT: to demonstrate wash-in (during late arterial phase) and washout (during portal venous and/or delayed venous phases). In addition, T2-weighted imaging, T1-weighted in- and out-phase imaging, and diffusion-weighted imaging (DWI) sequences are performed in order to assess ancillary features of HCC (Fig. 4.2).

MRI sensitivity is excellent for lesions with a size ≥2 cm and 1–2 cm (100% and 84% in a lesion-by-lesion analysis). However, sensitivity falls to 29–43% for lesions with a size <1 cm [26, 27]. In tumors with a size between 1 cm and 2 cm, MRI was shown to be superior over CT (sensitivity: 84% vs 47% for 1–2 cm) [28]. In clinical practice, the choice between imaging modality depends on institutional preferences and patient-specific factors.

Compared to CT, MRI provides higher contrast resolution and assessment of a greater number of tissue properties, which are valuable for lesion detection and characterization. However, MRI is limited by accessibility, longer acquisition time

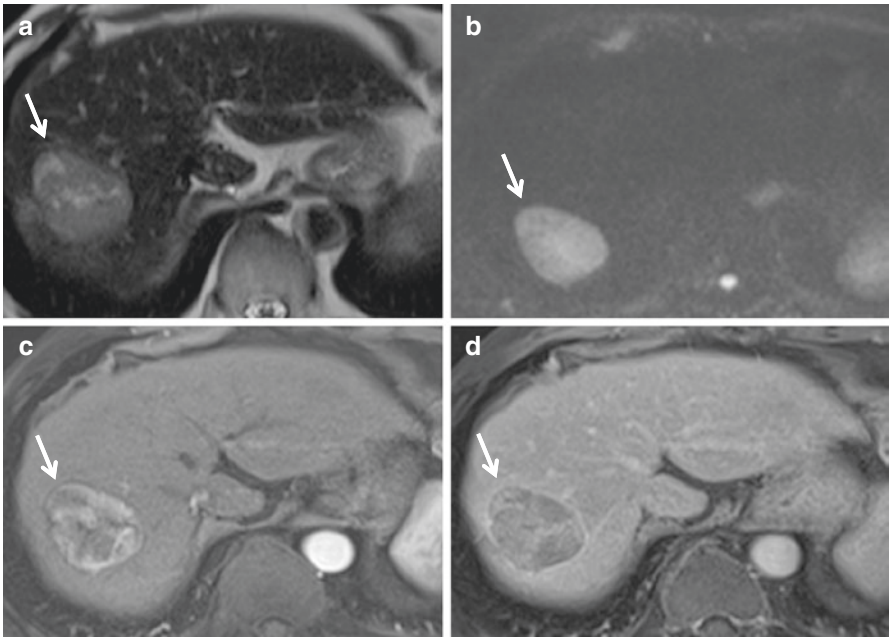

Fig. 4.2 A 75-year-old male patient with chronic hepatitis B virus cirrhosis and HCC. (**a**) Axial T2-weighted image demonstrates a moderately hyperintense lesion (arrow) in right hepatic lobe, with diffusion restriction on DWI (hyperintensity on high b-value image b800, **b**, arrow), heterogenous arterial hyperenhancement (wash-in) during the arterial phase (**c**, arrow) obtained with an extracellular gadolinium contrast agent (gadopentetate dimeglumine), and washout during late venous phase with enhancing capsule (**d**, arrow)

(30 min for a standard liver MRI protocol), the need for optimization and expertise to be accurately interpreted, and increased sensitivity to motion [25].

CT and MRI have both limited detection of well-differentiated and small HCCs. Furthermore, approximately 40% of HCC are not hypervascular during the arterial phase, including early HCC, infiltrative HCC, and some poorly differentiated HCC, and the presence of washout can be absent in approximately 40–60% of small HCC [29].

MRI with Liver-Specific Agents

Two different liver-specific contrast agents are commercially available in the United States: gadoxetic acid (or Gd-EOB-DTPA, FDA approved in 2008) and gadobenate dimeglumine (FDA approved in 2004). They mainly differ by the rate of biliary excretion (50% for gadoxetic acid and 3–5% for gadobenate dimeglumine) and the timing of the hepatobiliary phase (at 10–20 min versus 45 min–3 h after injection for gadoxetic acid versus gadobenate dimeglumine, respectively). For these reasons, Gd-EOB-DTPA is the agent most currently used for liver MRI protocols. The use of gadoxetic acid has increased in cirrhotic patients. Gadoxetic acid-enhanced MRI has been shown to be more sensitive than CT [30], particularly when considering early HCC. The data comparing ECCM vs gadoxetic acid is very limited. Lesion conspicuity and lesion-to-liver contrast ratio had shown to be significantly higher at hepatobiliary phase (HBP) compared to arterial phase images [31]. Gadoxetic acid allows assessment of both the vascular compartment and hepatobiliary function. When first injected, gadoxetic acid circulates through the vascular system for the acquisition of dynamic contrast phases. After approximately 5 min in healthy liver (longer in cirrhotic liver), around 50% of injected gadoxetic acid dose is taken up by functioning hepatocytes via the OATP8 transporter and subsequently excreted into the biliary system through MRP2 and MRP3 transporters. In a subsequent image acquisition at 10 and 20 min after contrast injection, labeled HBP or hepatocyte phase demonstrates liver contrast uptake with hyperintense liver parenchyma. Cirrhotic and low-grade dysplastic nodules may still express the OATP8 transporter; however with the progression to carcinogenesis within a cirrhotic nodule, the expression of OATP8 will decline. Thus, most HCC including some high-grade dysplastic nodules (HGDN) will appear hypointense during the HBP (Fig. 4.3). However, up to 10% of HCC may demonstrate some degree of hyperintensity on the HBP (due to residual OATP8 expression).

The HBP is valuable for characterizing small hypervascular lesions in the arterial phase without evidence of washout. On HBP, malignant lesions (in the context of cirrhosis, mainly HCC or less likely cholangiocarcinoma) will appear hypointense, while pseudolesions such as arterioportal shunts are isointense relative to surrounding liver parenchyma. For lesions that are hyperintense on HBP, ancillary image features assessed on other sequences such as T2-weighted imaging and DWI must be taken into account for distinguishing HCC from benign lesions such as focal nodular hyperplasia from hyperintense HCC.

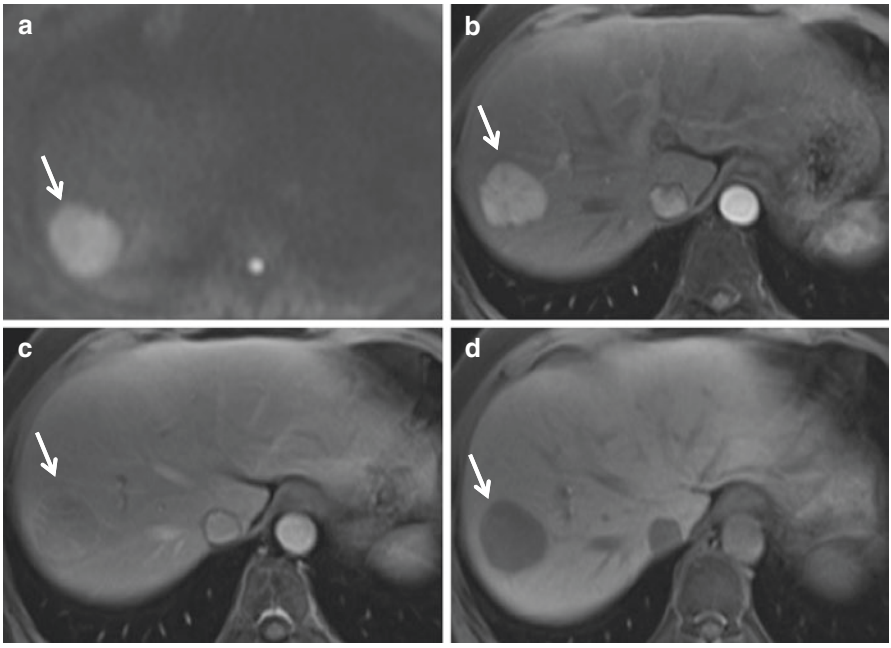

Fig. 4.3 A 72-year-old male noncirrhotic patient with chronic hepatitis B virus infection and HCC. DWI demonstrates right hepatic lobe lesion with diffusion restriction (**a**, hyperintense on high b-value image b800, arrow). Axial T1-weighted image demonstrates strong arterial hyperenhancement (wash-in) during arterial phase (**b**, arrow), washout during portal venous phase with capsule enhancement (**c**, arrow), and hypointensity at hepatobiliary phase post-gadoxetic acid injection (**d**, arrow), diagnostic for HCC in the setting of chronic liver disease

Compared to ECCM, gadoxetic acid has several limitations:

- Multiple North American studies have shown that the quality of the dynamic phases using gadoxetic acid may be limited by motion artifacts and sometimes breathing difficulties (called transient severe motion), which are exaggerated in comparison with ECCM. It has been described in up to 17% of MRI examinations [32].
- Dynamic imaging quality is limited due to the relatively smaller amount of gadolinium injected (0.025 mmol/kg for Gd-EOB-DTPA compared to 0.1 mmol/kg for ECCM) [33]. The trend is to inject a fixed dose of 10 mL in several institutions. The late venous phase acquired between 3 and 5 min post-contrast injection can be limited as hepatocyte uptake of contrast may have started, confounding assessment of washout at this time [34].
- Detection of HCC is challenging in the setting of advanced cirrhosis. Extensive hepatic fibrosis can appear hypointense on HBP. In the setting of severe liver dysfunction, cholestasis, or biliary obstruction, hepatocyte contrast uptake can be significantly reduced or absent. In this setting, MRI with ECCM is preferred.
- The cost of gadoxetic acid is higher compared to ECCM, in addition to the longer acquisition time.

Positron Emission Tomography-CT and MRI

^{18}Fluorodeoxyglucose (FDG) positron emission tomography (PET) is not included in the HCC diagnosis guidelines due to low sensitivity for detection (between 50% and 68%) [1, 7]. Sensitivity is better in poorly differentiated tumor due to a higher rate of FDG uptake in these tumors. Consequently, FDG uptake has been shown to be a prognostic marker for poorly differentiated tumor, microvascular invasion, shorter recurrence-free survival after curative treatment and short survival in case of palliative condition [35]. The role of FDG-PET for the evaluation of extrahepatic disease is also limited by low sensitivity, and current recommendations endorse CT, MRI, chest CT, and bone scintigraphy for this purpose. Dual tracer imaging with addition of ^{11}C-acetate or ^{18}F-choline is of interest in order to achieve a higher sensitivity and is currently under investigation [36]. With the emergence of quantitative imaging, the use of PET/MRI hybrid systems is promising [35, 37, 38].

Imaging Characteristics

Diagnostic Imaging Features

A distinction is currently made between early and progressed HCCs based on their pathological evolution. Early HCC can be considered as a "microinvasive carcinoma," while progressed HCC is a malignant neoplasm with ability to invade vessels and metastasize [24]. Consequently, imaging appearance of both HCC will differ. HCC imaging diagnosis features are mainly predicated on differences in vascularity between the lesion and background liver on dynamic contrast-enhanced imaging, as described above. Approximately 75% of the blood supply to the liver parenchyma is supplied by the portal vein, and 25% is supplied from the hepatic artery. During pathologic development from early to progressed HCC, portal flow into the tumor will decrease, while the proliferation of unpaired arteries and sinusoidal capillarization will result in an increase in hepatic arterial flow. Consequently, in late arterial phase imaging, HCC will appear hyperdense/intense compared to liver parenchyma (wash-in). Due to the decreased portal venous supply and arterioportal shunt within the tumor, the lesion will demonstrate washout in the portal venous and/or delayed venous phases. Wash-in is characteristic of progressed HCC while HGDN/early HCC may be iso−/hypovascular during the arterial phase. Wash-in appearance is sensitive for progressed HCC but not specific as several benign and malignant lesions can demonstrate arterial hyperenhancement, such as arterioportal shunt, hemangioma, focal nodular hyperplasia, focal fibrosis, cholangiocarcinoma, or hypervascular metastasis. Washout is the hypoenhancement of the tumor in comparison with the surrounding liver parenchyma in the portal venous and/or delayed venous phases. The explanation for washout is not completely understood. It is

probably multifactorial and due in part to early venous drainage, reduction of portal venous blood flow, and progressive enhancement of the liver parenchyma. As for the wash-in, washout in itself is not specific for HCC as it can be seen in cirrhotic liver in DN, cholangiocarcinoma, and biphenotypic HCC-cholangiocarcinoma. Nevertheless, in the setting of cirrhosis, a focal liver lesion >1 cm presenting wash-in and washout *is* an HCC (sensitivity of 100% in lesion with a size ≥2 cm and 90% in lesion with a size between 1 cm and 2 cm) according to AASLD criteria. Progressed HCC can also demonstrate a capsule/pseudocapsule surrounding the tumor. This is better visualized during the portal venous or late venous phases. Histologically, it is not always correlated with the presence of a real capsule, but it is specific of progressed HCC that makes it an important image feature for HCC diagnosis. In patients without history of liver disease, alternative diagnoses such as hepatocellular adenoma or hypervascular metastasis have to be considered as these lesions can have a similar imaging appearance.

Ancillary Imaging Features

When the tumor progresses, local invasion beyond the lesion is frequent and is manifested by the presence of satellite nodules surrounding the tumor. The presence of macrovascular invasion of the portal vein branches and/or hepatic veins needs to be assessed and should be part of the radiology report. Patients with cirrhosis and portal hypertension are at risk of bland venous thrombosis, which should be differentiated from a tumor thrombus. Thrombus location relative to the tumor and internal enhancement are key features to diagnose a tumor thrombus.

Other ancillary imaging features can aid in the diagnosis of HCC, although these are non-specific. The presence of intralesional fat is characteristic of early HCC. However, fat can also be present in DN, and therefore a fat-containing lesion can be considered as either malignant or premalignant. Corona enhancement, defined as the enhancement of the venous drainage area surrounding the tumor, is suggestive of hypervascular progressed HCC. Nodule-in-nodule appearance refers to the presence of a suspicious nodule within a larger nodule that histologically indicates the development of an HCC within a DN. Mosaic architecture refers to the presence of discrete internal compartments with differential enhancement and is characteristic of large HCC. Mosaic architecture is believed to reflect tumor heterogeneity and when present may be useful for differentiation of HCC from intrahepatic cholangiocarcinoma. The presence of mild to moderate T2 hyperintensity on T2 weighted imaging and DWI in the setting of chronic liver disease is specific for HCC. Focal lesional sparing in the setting of either diffuse hepatic iron deposition or steatosis can also be suggestive of HCC. Evidence of significant growth within 6 months is also an argument for malignancy. Finally, with the growing use of liver-specific agents for HCC detection, hypointensity at the HBP phase has been recently added as an ancillary feature suggestive of malignancy. In contrast, a lesion demonstrating either reduction in size or stability over ≥2 years,

enhancement pattern that follows blood pool, presence of undistorted vessels in the tumor vicinity, iron within a mass, marked T2 hyperintensity, and isointensity on HBP are all ancillary features favoring benignity.

Staging

HCC staging is critical for guiding treatment strategy. Among the different HCC staging systems, the Barcelona clinic liver cancer (BCLC) criteria are widely used as they integrate both relevant imaging findings and clinical factors such as underlying liver function and patient functional status into a therapeutic algorithm for each tumor stage, linking tumor stage with management strategy ranging from curative intent to supportive care [39]. Tumor staging – mainly based on lesion size and number and presence of macrovascular invasion – is based on radiologic appearance made on CT or MRI. The Organ Procurement and Transplantation Network (OPTN) also sets forth strict imaging criteria that are tied to tumor stage in order to determine eligibility and priority assignment for liver transplantation. Patients with one lesion up to 5 cm or two to three lesions up to 3 cm are eligible; however, patients with macrovascular invasion or evidence of extrahepatic disease are not eligible for liver transplantation [2].

Quantitative Imaging in HCC

Given the noninvasive imaging criteria for HCC, patients with typical imaging features of HCC can be diagnosed and treated without histopathologic confirmation [26]. However tissue sampling may provide information on molecular subtyping which provides valuable information on tumor aggressiveness [40]. There is growing interest in the use of imaging markers to provide tissue quantification as a surrogate of histopathologic findings. The introduction of molecular targeted agents for treatment of advanced stage HCC has also fueled interest in quantitative imaging markers of response assessment. The ultimate goal of quantitative imaging techniques is to achieve a personalized approach to cancer treatment response by identifying imaging biomarkers that will characterize tumor aggressiveness and potentially drive treatment decisions.

CT and MR Perfusion

CT and MR perfusion share the same principle of quantifying blood flow characteristics of focal liver lesions and background liver. Knowing the particular property of vascular modifications in the HCC tumorigenesis helps identify the changes from a

portal venous predominant blood supply to an arterialized flow. Perfusion measures the variation of contrast concentration in a determined tissue during time. For the calculation, the tissue of interest is scanned repeatedly before, during, and after contract injection. The schematic tissue enhancement can be divided in two phases according to the space where the contrast is located: the intravascular and the extravascular extracellular spaces. The use of a vascular input function (in the aorta used as a surrogate for the hepatic artery and the portal vein) and a pharmacokinetic model [41] allows the quantification of perfusion parameters such as blood flow, blood volume, mean transit time, permeability, hepatic arterial perfusion, portal venous perfusion, and hepatic perfusion index [42–45]. Additionally, model-free parameters can be calculated such as slope or area under the curve to described tumor or liver parenchyma time/concentration curve [43, 46]. For all parameters, parametric maps can be generated. These parameters can be conceived to reflect physiologic markers related to tumor angiogenesis.

Perfusion imaging can be acquired with both CT and MRI. Perfusion CT is limited by radiation exposure due to the repetitive scanning requirement. The advantage with perfusion MRI is that it can be combined to other functional imaging as DWI in the same MRI acquisition. Both techniques are limited to the volume of tissue coverage and suffer from a need of standardized protocols, variable repeatability and inter-platform reproducibility [47].

Due to the HCC vascular profile (neoangiogenesis with development of arteriolar network), perfusion parameters in HCC lesions are different from adjacent liver parenchyma [48] (Fig. 4.4). However, there are possible differences in perfusion parameters related to tumor grade [49]. Perfusion imaging can be also used for assessment of tumor response after locoregional therapy, such as transarterial chemoembolization (TACE) [45]. Persistence or apparition of arterial perfusion after TACE corresponds to incomplete treatment or tumor recurrence [50]. Perfusion for the assessment of early response to therapy such as TACE and Yttrium90 radioembolization or emerging molecular targeted agents is also promising for better selection of patients [51, 52].

Dual Energy CT

Dual energy CT (DECT) is a technique based on the different elemental composition of the tissue. Compared to conventional CT, DECT allows differentiation of material in tissue composition based on differences in iodine and water densities. DECT allows selective quantification and visualization of iodine-related density differences by providing polychromatic images using two orthogonal X-ray tubes working at low and high voltages (80 kVp and 140 kVp, respectively) [53]. This method enables virtual reconstructed images using a combination of images acquired at the low- and high-energy acquisitions and allows reconstruction of virtual unenhanced images. DECT improves the ability to distinguish high-density

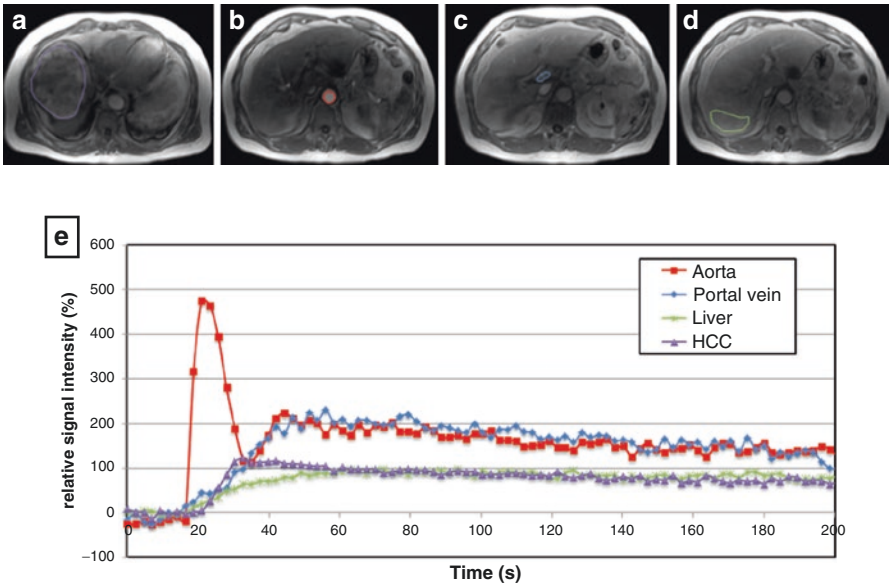

Fig. 4.4 A 66-year-old male patient with large HCC in the right hepatic lobe (13 cm). Axial DCE-MR images acquired using 3D-FLASH sequence demonstrate ROI placement in different regions at different time points: tumor (*purple*) acquired at 20 s postinjection (**a**), abdominal aorta (*red*) (**b**, 11 s postinjection), main portal vein (*blue*) (**c**, 28 s postinjection), and liver parenchyma (*green*) (**d**, 55 s postinjection). Plots below (**e**) demonstrate relative signal intensity versus time curve [*y*-axis: 100× ($SI/SI_0 - 1$), with SI and SI_0 representing the signal intensity and signal intensity before enhancement, respectively]. Plot shows fast enhancement and subsequent washout of HCC as opposed to slow enhancement of the liver parenchyma. (This figure has been reproduced with the permission from Springer Nature)

substances created by iodine (enhancement) from those created by hemorrhage and to detect subtle enhancement.

Furthermore, selective iodine-related attenuation and volumetric iodine uptake in a specific tissue can be measured, enabling quantification of tissue perfusion by providing iodine maps and information regarding blood volume. As the amount of iodinated contrast medium in tissue depends on its degree of vascularization, the amount of volume iodine-intake may be considered as representative of blood perfusion and vascularization in the tumor [54]. It has been described as a surrogate to perfusion CT allowing a reduction in radiation dose [55].

In clinical practice for HCC, dual energy CT is used as an alternative to conventional multiphasic CT in order to reduce radiation dose as it allows virtual non-contrast acquisition by identifying and subtracting the iodine component of an enhanced phase [56]. DECT has shown efficacy for HCC detection and characterization using both qualitative (lesion conspicuity) and quantitative (using iodine density values) image analysis [57].

Diffusion-Weighted Imaging

DWI is a non-contrast MRI sequence that quantifies the motility of water protons within the tissue and provides information on tissue cellularity and integrity of the cell membranes [58]. High cellularity (as in tumors), distortion of the extracellular space (as in cirrhosis), and increased density of hydrophobic membranes within tissues restrict water diffusion. In hypercellular tissue, extracellular water cannot diffuse, and this results in a reduction on the apparent diffusion coefficient (ADC). A cystic/necrotic component has few structures to restrict diffusion, and this results in a high ADC [58]. ADC contains information reflecting a combination of cellular density and perfusion (microcirculation) and is derived from the monoexponential fitting of the signal intensity decay curve. DWI is used in daily clinical practice for liver lesion detection and characterization [15]. DWI has generally shown equal to superior performance compared to T2-weighted images and is helpful when employed in conjunction with contrast-enhanced sequences. It is also of a great interest for patients with contraindications to gadolinium-based contrast agents. Based on their composition, cysts and hemangiomas can typically be distinguished from solid liver lesions with reasonable accuracy, with some degree of overlap [59]. ADC also increases after locoregional therapy (LRT) and systemic therapy, correlating with necrotic changes in response to therapy [60, 61].

Intravoxel incoherent motion (IVIM) DWI captures relative contributions of true cellular diffusion and microvascular perfusion within a tissue, which are affected by several physiologic and pathophysiologic factors, including the presence of restrictive barriers within the tissue, fluid viscosity, and fractional volume of perfusing spins [62]. These characteristics may enable IVIM to detect and characterize the tissue changes caused by disease. As opposed to DWI, IVIM is derived from biexponential fitting of the signal intensity decay curve. IVIM calculation allowed to obtain different parameters: perfusion fraction (PF or *f*) is the fraction of pseudodiffusion, Dslow (or D_t or D) is the true diffusion coefficient representing the pure molecular diffusion, and Dfast (or D∗) is the pseudodiffusion coefficient that means the incoherent microcirculation within the voxel [62]. Limitations of standard DWI and IVIM suffer from a low spatial resolution and high susceptibility to artifact: heart motion artifact (and other motion as peristaltic bowel) and susceptibility artifact at the boundary surfaces.

IVIM has shown promise for the diagnosis of liver fibrosis/cirrhosis diagnosis [63] and assessment of response to therapy [64] (Fig. 4.5). However, data on HCC response using IVIM is limited.

Radiomics Quantification

In the setting of translational and precision medicine, radiomics and radiogenomics represent more advanced steps of research in the field in radiology. Radiomics is based on the concept that images contain information about pathophysiology that

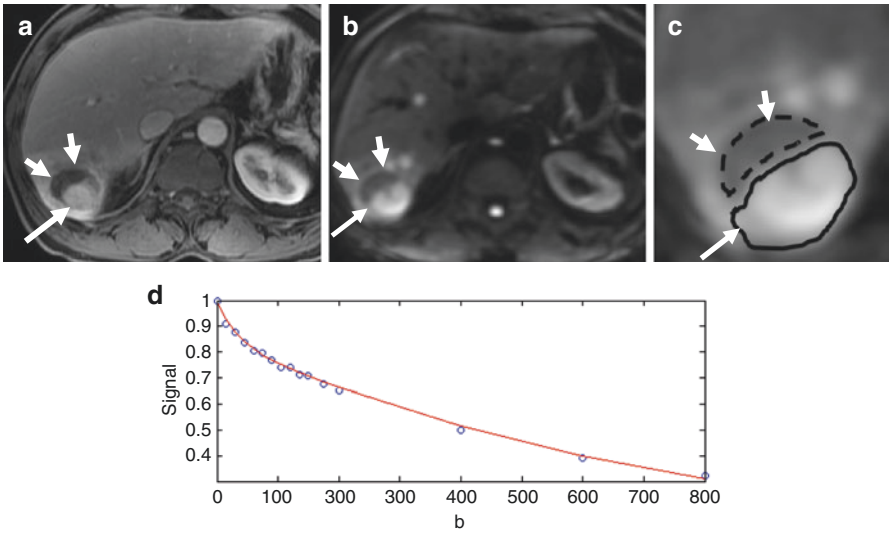

Fig. 4.5 A 59-year-old male patient with partially necrotic HCC post TACE. (**a**) Axial contrast-enhanced T1 weighted image obtained during portal venous phase, (**b**) axial fat suppressed DWI image at b800. Both demonstrate a partially necrotic HCC with solid enhancing component with restricted diffusion (arrow) and non-enhancing necrotic component which is hypointense on DWI (short arrows). (**c**) Magnified b800 DW images show ROI placement in solid and necrotic components. (**d**) Biexponential diffusion modeling for quantification of diffusion and perfusion (IVIM: intravoxel incoherent motion DWI) in the whole HCC lesion. The following parameters were obtained in a solid/necrotic HCC components/background liver: D 1.21/1.57.0.77 × 10^{-3} mm^2/s, PF 15.81%/23.33%/21.58%, ADC 1.47/1.97/1.09 × 10^{-3} mm^2/s, and enhancement ratios (during portal venous phase) 113.0%/−16.4%/47.8%. (Figure copyright S. Kakite, licensed under CC BY-NC-ND 4.0)

can be expressed by the extraction of a large number of quantitative features. Radiomics analysis provides a large amount of data that has then to be processed, statistically analyzed, pooled, compared, and integrated into a clinical situation. Radiomics has been designed in order to be a decision support tool, giving information on disease detection, diagnosis, prognosis, and response to therapy. Cancer imaging follow-up allows potentially an indefinite amount of data for each patient with repetitive follow-up in the course of his or her cancer. An essential step of the process to the application of radiomics in clinical care is to create large database pooling and comparing the results of research groups from different centers in order to get solid and reproducible datasets [65, 66].

Going one step beyond, radiogenomics is the correlation of radiomics data with gene signatures and gene expression profiles. Recent studies suggest that radiomics features may reflect biological processes occurring at the genetic and molecular level [67]. Compared to the histopathologic and genetic analysis of tumor tissue (obtained either by biopsy or following tumor resection), radiomics and radiogenomics analysis offers several advantages. First, an analysis of the entire tumor can be performed by radiomics analysis compared to biopsy. Second,

intra-tumoral heterogeneity limits the usefulness of tissue sampling by biopsy performance that is known to have a sampling error rate as high as 20.3% [68, 69]. Radiomics can be used to quantify tumor heterogeneity to predict the best target location for a biopsy in order to reduce the error rate. Third, it is well known that genetic expression changes during time due to cancer evolution and response to therapy. When repeated biopsies are not acceptable, repeat radiomics analysis is feasible during cancer follow-up.

Qualitative and quantitative features based on intensity, shape, size, volume, or texture can provide information about tumor phenotype and microenvironment that can be correlated to clinical outcomes.

Radiomics requires different steps: (1) image acquisition, (2) identification of the volume of interest, (3) volume segmentation, (4) extraction and qualification of descriptive features from the volume, (5) using these data to conceive and enrich a database, and (6) using the database to develop model classifiers to predict outcome. Each step has its limitations and challenges.

Medical images are acquired using a wide range of acquisition parameters and reconstruction protocols that may have a substantial effect on image quantification with radiomics analysis. An effort of standardization is currently performed by the professional societies as, for instance, the American College of Radiology and the Radiological Society of North America (RSNA) that promote guidelines on quantitative imaging.

Radiomics in HCC has shown early interest in the technique development. Zhou et al. studied the mean intensity and gray-level run-length nonuniformity (a texture feature) of the MRI arterial phase of 46 patients with resected HCC. Based on these features, they were able to predict HCC tumor grade (high versus low grade) with a sensitivity of 76% and a specificity of 100% [70]. Multiple texture features have been shown to predict microvascular invasion in HCC with good accuracy [71]. Segal et al. reconstructed 78% of the global gene expression profile of HCC by combining 28 distinct imaging traits on CT. These traits were linked to cell proliferation, liver synthetic function, and prognosis [72]. Correlation has also been made between CT and MRI features and gene signature of aggressive HCC [73, 74]. An example of quantification of HCC heterogeneity is shown in Fig. 4.6. Radiomics limitations relate to reproducibility and standardization. It is also challenging to adequately manage large amount of data.

Current imaging strategies are not able to differentiate the two causes of HCC multifocality: intrahepatic metastasis and multicentric carcinogenesis. These two different pathways are known to have different prognosis. In the future, radiomics may be able to determine this difference, which may have direct consequences on HCC staging systems and treatment.

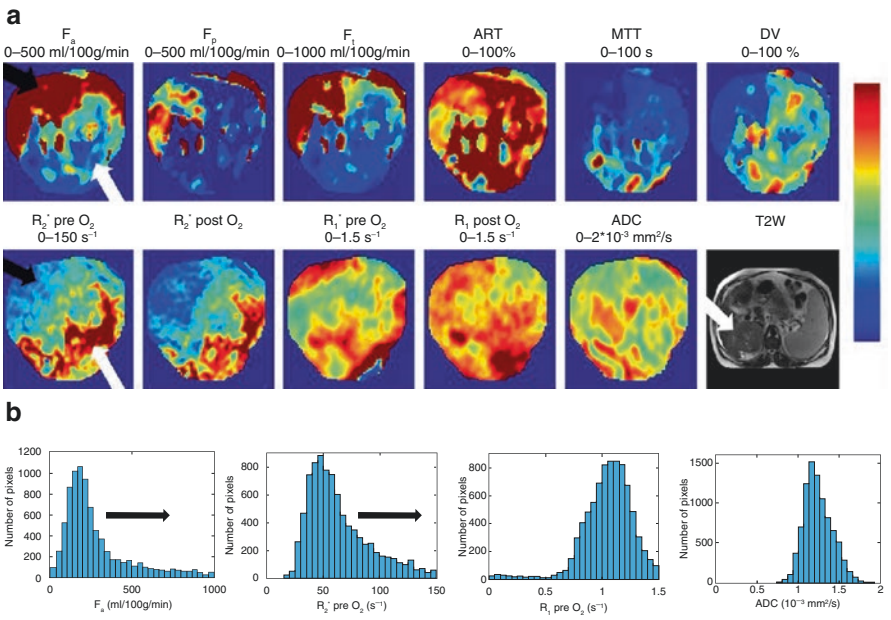

Fig. 4.6 A 54-year-old male patient with chronic hepatitis B virus cirrhosis and HCC. (**a**) Representative magnified parametric maps of a large (8.3 cm) HCC. Location of the tumor within the liver is indicated by the white arrow on the T2-weighted image (bottom row, right). A distinct region in the anterior portion of the tumor of high arterial flow (Fa) and low R2∗ was observed, reflective of high tumor perfusion and normoxia (gray arrow in Fa and R2∗ pre O2 maps). The posterior portion of the tumor displays low Fa and high R2∗, suggestive of poor perfusion and hypoxia (white arrow in Fa and R2∗ pre O2 maps). (**b**) Histograms of Fa, R2∗ pre O2, R1 pre O2, and ADC in the same lesion. The extensive heterogeneity observed in the parameter maps of Fa and R2∗ pre O2 is also reflected in the histograms, as illustrated by the fat tails and pronounced skewness, indicated by arrows. The R1 pre O2 histogram also exhibited skewness (black arrow). ADC apparent diffusion coefficient, ART arterial fraction, DV distribution volume, Fa arterial flow, Fp portal flow, Ft total flow, MTT mean transit time, R1 longitudinal relaxation rate, R2∗ transverse relaxation rate. (Figure copyright S. Hectors, licensed under CC BY 4.0)

Conclusion

Imaging is central in HCC diagnosis and management. With its vascular characteristics, typical HCC can be diagnosed with imaging without the need for histopathologic confirmation. Tissue sampling may be needed in atypical cases and for the purpose of molecular profiling. Additional advantages of imaging include (1) the

possibility of analyzing the entire lesion and enabling lesion heterogeneity analysis and (2) repeat imaging assessments that can be performed for assessing response to therapy. Emerging quantitative imaging techniques such as radiomics may enable better tumor characterization and assessment of tumor aggressiveness, which may help personalize therapy in patients with HCC.

References

1. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2.
2. Wald C, Russo MW, Heimbach JK, Hussain HK, Pomfret EA, Bruix J. New OPTN/UNOS policy for liver transplant allocation: standardization of liver imaging, diagnosis, classification, and reporting of hepatocellular carcinoma. Radiology. 2013;266(2):376–82.
3. Durand F, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, et al. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. J Hepatol. 2001;35(2):254–8.
4. Shyamala K, Girish HC, Murgod S. Risk of tumor cell seeding through biopsy and aspiration cytology. J Int Soc Prev Community Dent. 2014;4(1):5–11.
5. Kramer H, Pickhardt PJ, Kliewer MA, Hernando D, Chen G-H, Zagzebski JA, et al. Accuracy of liver fat quantification with advanced CT, MRI, and ultrasound techniques: prospective comparison with MR spectroscopy. AJR Am J Roentgenol. 2017;208(1):92–100.
6. Grgurevic I, Puljiz Z, Brnic D, Bokun T, Heinzl R, Lukic A, et al. Liver and spleen stiffness and their ratio assessed by real-time two dimensional-shear wave elastography in patients with liver fibrosis and cirrhosis due to chronic viral hepatitis. Eur Radiol. 2015;25(11):3214–21.
7. European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer. EASL–EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43.
8. International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology. 2009;49(2):658–64.
9. Omata M, Lesmana LA, Tateishi R, Chen P-J, Lin S-M, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010;4(2):439–74.
10. Kudo M, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, et al. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. Dig Dis. 2011;29(3):339–64.
11. Giannini EG, Cucchetti A, Erroi V, Garuti F, Odaldi F, Trevisani F. Surveillance for early diagnosis of hepatocellular carcinoma: how best to do it? World J Gastroenterol. 2013;19(47):8808–21.
12. LI-RADS [Internet]. [cited 2018 Aug 21]. Available from: https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS.
13. Santillan CS, Tang A, Cruite I, Shah A, Sirlin CB. Understanding LI-RADS: a primer for practical use. Magn Reson Imaging Clin N Am. 2014;22(3):337–52.
14. Darnell A, Forner A, Rimola J, Reig M, García-Criado Á, Ayuso C, et al. Liver imaging reporting and data system with MR imaging: evaluation in nodules 20 mm or smaller detected in cirrhosis at screening US. Radiology. 2015;275(3):698–707.
15. CT/MRI LI-RADS v2018 [Internet]. [cited 2018 Aug 10]. Available from: https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS/CT-MRI-LI-RADS-v2018.
16. Bolondi L. Screening for hepatocellular carcinoma in cirrhosis. J Hepatol. 2003;39(6):1076–84.

17. Kim SY, An J, Lim Y-S, Han S, Lee J-Y, Byun JH, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. JAMA Oncol. 2017;3(4):456–63.
18. Dodd GD, Miller WJ, Baron RL, Skolnick ML, Campbell WL. Detection of malignant tumors in end-stage cirrhotic livers: efficacy of sonography as a screening technique. AJR Am J Roentgenol. 1992;159(4):727–33.
19. Nowicki TK, Markiet K, Szurowska E. Diagnostic imaging of hepatocellular carcinoma – a pictorial essay. Curr Med Imaging Rev. 2017;13(2):140–53.
20. Maruyama H, Takahashi M, Ishibashi H, Yoshikawa M, Yokosuka O. Contrast-enhanced ultrasound for characterisation of hepatic lesions appearing non-hypervascular on CT in chronic liver diseases. Br J Radiol. 2012;85(1012):351–7.
21. Takahashi M, Maruyama H, Shimada T, Kamezaki H, Sekimoto T, Kanai F, et al. Characterization of hepatic lesions (≤30 mm) with liver-specific contrast agents: a comparison between ultrasound and magnetic resonance imaging. Eur J Radiol. 2013;82(1):75–84.
22. Sugimoto K, Moriyasu F, Shiraishi J, Saito K, Taira J, Saguchi T, et al. Assessment of arterial hypervascularity of hepatocellular carcinoma: comparison of contrast-enhanced US and gadoxetate disodium-enhanced MR imaging. Eur Radiol. 2012;22(6):1205–13.
23. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsøe CP, et al. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver – update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. Ultrasound Med Biol. 2013;39(2):187–210.
24. Choi J-Y, Lee J-M, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part I. Development, growth, and spread: key pathologic and imaging aspects. Radiology. 2014;272(3):635–54.
25. Choi J-Y, Lee J-M, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part II. Extracellular agents, hepatobiliary agents, and ancillary imaging features. Radiology. 2014;273(1):30–50.
26. Sangiovanni A, Manini MA, Iavarone M, Romeo R, Forzenigo LV, Fraquelli M, et al. The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. Gut. 2010;59(5):638–44.
27. Rode A, Bancel B, Douek P, Chevallier M, Vilgrain V, Picaud G, et al. Small nodule detection in cirrhotic livers: evaluation with US, spiral CT, and MRI and correlation with pathologic examination of explanted liver. J Comput Assist Tomogr. 2001;25(3):327–36.
28. Burrel M, Llovet JM, Ayuso C, Iglesias C, Sala M, Miquel R, et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology. 2003;38(4):1034–42.
29. Khan AS, Hussain HK, Johnson TD, Weadock WJ, Pelletier SJ, Marrero JA. Value of delayed hypointensity and delayed enhancing rim in magnetic resonance imaging diagnosis of small hepatocellular carcinoma in the cirrhotic liver. J Magn Reson Imaging: JMRI. 2010;32(2):360–6.
30. Kim BR, Lee JM, Lee DH, Yoon JH, Hur BY, Suh KS, et al. Diagnostic performance of gadoxetic acid–enhanced liver MR imaging versus multidetector CT in the detection of dysplastic nodules and early hepatocellular carcinoma. Radiology. 2017;285(1):134–46.
31. Besa C, Kakite S, Cooper N, Facciuto M, Taouli B. Comparison of gadoxetic acid and gadopentetate dimeglumine-enhanced MRI for HCC detection: prospective crossover study at 3 T. Acta Radiol Open. 2015;4(2):2047981614561285.
32. Davenport MS, Viglianti BL, Al-Hawary MM, Caoili EM, Kaza RK, Liu PSC, et al. Comparison of acute transient dyspnea after intravenous administration of gadoxetate disodium and gadobenate dimeglumine: effect on arterial phase image quality. Radiology. 2013;266(2):452–61.
33. Tanimoto A, Higuchi N, Ueno A. Reduction of ringing artifacts in the arterial phase of gadoxetic acid-enhanced dynamic MR imaging. Magn Reson Med Sci: MRMS. 2012;11(2):91–7.

34. Cruite I, Schroeder M, Merkle EM, Sirlin CB. Gadoxetate disodium–enhanced MRI of the liver: part 2, protocol optimization and lesion appearance in the cirrhotic liver. Am J Roentgenol. 2010;195(1):29–41.
35. Cho KJ, Choi NK, Shin MH, Chong AR. Clinical usefulness of FDG-PET in patients with hepatocellular carcinoma undergoing surgical resection. Ann Hepatobiliary Pancreat Surg. 2017;21(4):194–8.
36. Haug AR. Imaging of primary liver tumors with positron-emission tomography. Q J Nucl Med Mol Imaging. 2017;61(3):292–300.
37. Kong E, Chun KA, Cho IH. Quantitative assessment of simultaneous F-18 FDG PET/MRI in patients with various types of hepatic tumors: correlation between glucose metabolism and apparent diffusion coefficient. PLoS One [Internet]. 2017 [cited 2018 Jul 5];12(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5495334/.
38. Hectors S, Wagner M, Besa C, Huang W, Taouli B. Multiparametric FDG-PET/MRI of hepatocellular carcinoma: initial experience. Contrast Media Mol Imaging. 2018;2018:5638283.
39. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19(03):329–38.
40. Torbenson M, Schirmacher P. Liver cancer biopsy – back to the future?!. Hepatology. 2015;61(2):431–3.
41. Materne R, Smith AM, Peeters F, Dehoux JP, Keyeux A, Horsmans Y, et al. Assessment of hepatic perfusion parameters with dynamic MRI. Magn Reson Med. 2002;47(1):135–42.
42. Kim SH, Kamaya A, Willmann JK. CT perfusion of the liver: principles and applications in oncology. Radiology. 2014;272(2):322–44.
43. Thng CH, Koh TS, Collins D, Koh D-M. Perfusion imaging in liver MRI. Magn Reson Imaging Clin N Am. 2014;22(3):417–32.
44. Hagiwara M, Rusinek H, Lee VS, Losada M, Bannan MA, Krinsky GA, et al. Advanced liver fibrosis: diagnosis with 3D whole-liver perfusion MR imaging--initial experience. Radiology. 2008;246(3):926–34.
45. Taouli B, Johnson RS, Hajdu CH, Oei MTH, Merad M, Yee H, et al. Hepatocellular carcinoma: perfusion quantification with dynamic contrast-enhanced MRI. AJR Am J Roentgenol. 2013;201(4):795–800.
46. Aronhime S, Calcagno C, Jajamovich GH, Dyvorne HA, Robson P, Dieterich D, et al. DCE-MRI of the liver: effect of linear and nonlinear conversions on hepatic perfusion quantification and reproducibility. J Magn Reson Imaging: JMRI. 2014;40(1):90–8.
47. Miles KA, Lee T-Y, Goh V, Klotz E, Cuenod C, Bisdas S, et al. Current status and guidelines for the assessment of tumour vascular support with dynamic contrast-enhanced computed tomography. Eur Radiol. 2012;22(7):1430–41.
48. Pandharipande PV, Krinsky GA, Rusinek H, Lee VS. Perfusion imaging of the liver: current challenges and future goals. Radiology. 2005;234(3):661–73.
49. Tarhan NC, Hatipoğlu T, Ercan E, Bener M, Keleş G, Başaran C, et al. Correlation of dynamic multidetector CT findings with pathological grades of hepatocellular carcinoma. Diagn Interv Radiol. 2011;17(4):328–33.
50. Yang L, Zhang XM, Zhou XP, Tang W, Guan YS, Zhai ZH, et al. Correlation between tumor perfusion and lipiodol deposition in hepatocellular carcinoma after transarterial chemoembolization. J Vasc Interv Radiol: JVIR. 2010;21(12):1841–6.
51. Nakamura Y, Kawaoka T, Higaki T, Fukumoto W, Honda Y, Iida M, et al. Hepatocellular carcinoma treated with sorafenib: arterial tumor perfusion in dynamic contrast-enhanced CT as early imaging biomarkers for survival. Eur J Radiol. 2018;98:41–9.
52. Tamandl D, Waneck F, Sieghart W, Unterhumer S, Kölblinger C, Baltzer P, et al. Early response evaluation using CT-perfusion one day after transarterial chemoembolization for HCC predicts treatment response and long-term disease control. Eur J Radiol. 2017;90:73–80.
53. Johnson TRC, Krauss B, Sedlmair M, Grasruck M, Bruder H, Morhard D, et al. Material differentiation by dual energy CT: initial experience. Eur Radiol. 2007;17(6):1510–7.

54. Apfaltrer P, Meyer M, Meier C, Henzler T, Barraza JM, Dinter DJ, et al. Contrast-enhanced dual-energy CT of gastrointestinal stromal tumors: is iodine-related attenuation a potential indicator of tumor response? Investig Radiol. 2012;47(1):65–70.
55. Gordic S, Puippe GD, Krauss B, Klotz E, Desbiolles L, Lesurtel M, et al. Correlation between dual-energy and perfusion CT in patients with hepatocellular carcinoma. Radiology. 2016;280(1):78–87.
56. Kaufmann S, Sauter A, Spira D, Gatidis S, Ketelsen D, Heuschmid M, et al. Tin-filter enhanced dual-energy-CT: image quality and accuracy of CT numbers in virtual noncontrast imaging. Acad Radiol. 2013;20(5):596–603.
57. Laroia ST, Bhadoria AS, Venigalla Y, Chibber GK, Bihari C, Rastogi A, et al. Role of dual energy spectral computed tomography in characterization of hepatocellular carcinoma: initial experience from a tertiary liver care institute. Eur J Radiol Open. 2016;3:162–71.
58. Saito K, Tajima Y, Harada TL. Diffusion-weighted imaging of the liver: current applications. World J Radiol. 2016;8(11):857–67.
59. Shenoy-Bhangle A, Baliyan V, Kordbacheh H, Guimaraes AR, Kambadakone A. Diffusion weighted magnetic resonance imaging of liver: principles, clinical applications and recent updates. World J Hepatol. 2017;9(26):1081–91.
60. Barat M, Fohlen A, Cassinotto C, Jannot AS, Dautry R, Pelage J-P, et al. One-month apparent diffusion coefficient correlates with response to radiofrequency ablation of hepatocellular carcinoma. J Magn Reson Imaging: JMRI. 2017;45(6):1648–58.
61. Mannelli L, Kim S, Hajdu CH, Babb JS, Taouli B. Serial diffusion-weighted MRI in patients with hepatocellular carcinoma: prediction and assessment of response to transarterial chemoembolization. Preliminary experience. Eur J Radiol. 2013;82(4):577–82.
62. Li YT, Cercueil J-P, Yuan J, Chen W, Loffroy R, Wáng YXJ. Liver intravoxel incoherent motion (IVIM) magnetic resonance imaging: a comprehensive review of published data on normal values and applications for fibrosis and tumor evaluation. Quant Imaging Med Surg. 2017;7(1):59–78.
63. Patel J, Sigmund EE, Rusinek H, Oei M, Babb JS, Taouli B. Diagnosis of cirrhosis with intravoxel incoherent motion diffusion MRI and dynamic contrast-enhanced MRI alone and in combination: preliminary experience. J Magn Reson Imaging: JMRI. 2010;31(3):589–600.
64. Kakite S, Dyvorne H, Besa C, Cooper N, Facciuto M, Donnerhack C, et al. Hepatocellular carcinoma: short-term reproducibility of apparent diffusion coefficient and intravoxel incoherent motion parameters at 3.0T. J Magn Reson Imaging: JMRI. 2015;41(1):149–56.
65. Gillies RJ, Kinahan PE, Hricak H. Radiomics: images are more than pictures, they are data. Radiology. 2016;278(2):563–77.
66. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: the bridge between medical imaging and personalized medicine. Nat Rev Clin Oncol. 2017;14(12):749–62.
67. Pinker K, Shitano F, Sala E, Do RK, Young RJ, Wibmer AG, et al. Background, current role, and potential applications of radiogenomics. J Magn Reson Imaging: JMRI. 2018;47(3):604–20.
68. Clauson J, Hsieh YC, Acharya S, Rademaker AW, Morrow M. Results of the Lynn Sage Second-Opinion Program for local therapy in patients with breast carcinoma. Changes in management and determinants of where care is delivered. Cancer. 2002;94(4):889–94.
69. Robert M, Sofair AN, Thomas A, Bell B, Bialek S, Corless C, et al. A comparison of hepatopathologists' and community pathologists' review of liver biopsy specimens from patients with hepatitis C. Clin Gastroenterol Hepatol. 2009;7(3):335–8.
70. Zhou W, Zhang L, Wang K, Chen S, Wang G, Liu Z, et al. Malignancy characterization of hepatocellular carcinomas based on texture analysis of contrast-enhanced MR images. J Magn Reson Imaging: JMRI. 2017;45(5):1476–84.
71. Peng J, Zhang J, Zhang Q, Xu Y, Zhou J, Liu L. A radiomics nomogram for preoperative prediction of microvascular invasion risk in hepatitis B virus-related hepatocellular carcinoma. Diagn Interv Radiol. 2018;24(3):121–7.

72. Segal E, Sirlin CB, Ooi C, Adler AS, Gollub J, Chen X, et al. Decoding global gene expression programs in liver cancer by noninvasive imaging. Nat Biotechnol. 2007;25(6):675–80.
73. Taouli B, Hoshida Y, Kakite S, Chen X, Tan PS, Sun X, et al. Imaging-based surrogate markers of transcriptome subclasses and signatures in hepatocellular carcinoma: preliminary results. Eur Radiol. 2017;27(11):4472–81.
74. Hectors SJ, Wagner M, Bane O, Besa C, Lewis S, Remark R, et al. Quantification of hepatocellular carcinoma heterogeneity with multiparametric magnetic resonance imaging. Sci Rep [Internet]. 2017 [cited 2018 Jun 12];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5446396/.

Chapter 5
Analysis of Hepatocellular Carcinoma Tissue for Biomarker Discovery

Connor A. West, Alyson P. Black, and Anand S. Mehta

Introduction

Liver cancer causes more than 700,000 deaths annually, making it the fifth most common cancer overall and second most common cause of cancer-related deaths worldwide [1, 2]. Hepatocellular carcinoma (HCC) is the most common form of liver cancer with multiple known risk factors, such as chronic hepatitis B virus (HBV), chronic infection with hepatitis C virus (HCV), alcohol abuse, obesity, and many other metabolic diseases [3]. These risk factors induce a progressive inflammatory response, resulting in liver fibrosis and eventually cirrhosis, which is the true risk factor for HCC. This process occurs in multiple cycles of necrosis and regeneration, often leading to genetic instability [4]. Because of this genetic heterogeneity, the pathways involved in hepatocarcinogenesis are not fully clear, resulting in a lack of diagnostic and therapeutic options [5]. Therefore the survival rates of primary liver cancer are low, generally with a 0.95 ratio of mortality to occurrence and 5-year survival rates as low as 11% [6, 7].

Over the last 10 years, HCC is the cancer with the greatest increase in mortality in the United States of America (USA). In the Annual Report to the Nation on the Status of Cancer, between 1975 and 2012, mortality from HCC increased at an annual rate of 2.8% in men and 2.2% in women [8]. Indeed, the occurrence of liver cancer is predicted to continue rising in the United States and will exceed 50,000 cases by the year 2021 and will be associated with greater mortality than observed with breast or colorectal cancer [9].

C. A. West · A. P. Black · A. S. Mehta (✉)
Department of Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA
e-mail: mehtaa@musc.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_5

Treatment Options

Related to the low survival rates of liver cancer patients, there are few treatments and even fewer curative options, especially for those patients with large lesions. While there are chemotherapeutic possibilities and ablation and resection techniques for lesions smaller than 3–5 cm in size, there are few curative options (survival greater than 60 months). These are surgical resection of small lesions and transplantation [10]. The patient's unique clinical case, which contains a variety of factors such as hepatic reserve, hepatic function, and lesion size, determines which method is most viable. By most standards, patients with fewer and smaller lesions, as well as ample hepatic reserves, are often good candidates for resection, with 5-year survival and disease-free survival rates at 39% and 26%, respectively [10, 11]. Resection, however, is usually available to only 10–37% of patients at the time of diagnosis [11], and transplantation availability is even lower. Transplantation is the most successful form of curative therapy for liver cancer patients with overall and disease-free survival rates at 85% and 92%, respectively, but complications from immune rejection and lack of organ donors for patients result in transplantations being less common as a treatment technique [10, 11].

Chemotherapeutic options for HCC are limited and used primarily in those who are not candidates for resection. The frontline agent for those with non-ablatable tumors is the multi-kinase inhibitor sorafenib, sold under the brand name Nexavar. Sorafenib is a general tyrosine and serine/threonine protein kinase inhibitor with activity against vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors as well as intracellular kinases B-Raf and Raf-1 [8]. Agents that specifically target one growth receptor, such as enhanced VEGFR inhibitors, have failed to show activity against HCC [8]. It is noted that sorafenib's activity against HCC is limited, with improved survival times of only a few months [9]. These bleak treatment options—both in their availability and efficacy—highlight the necessity for early detection of HCC.

Current Detection Methods

Current guidelines by the American Association for the Study of Liver Diseases (AASLD), National Comprehensive Cancer Network (NCCN), and Department of Veterans Affairs (VA) recommend HCC surveillance with abdominal US with or without α-fetoprotein (AFP) every 6 months in all patients with cirrhosis [11]. Although there is no randomized trial evaluating HCC surveillance in patients with cirrhosis, several prospective cohort studies have demonstrated an association between HCC surveillance and improvement in early detection and survival in patients with cirrhosis, after adjusting for known confounders and lead-time bias [12, 13]. Although the surveillance has efficacy, the majority of the patients in the USA are diagnosed beyond the early stage where curative therapies are no longer

effective. In addition to poor sensitivity for early HCC detection, US and AFP are both prone to false positive results, leading to unnecessary patient anxiety and diagnostic testing [14, 15]. While some providers use alternative, expensive imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI), in all cirrhosis patients (despite a dearth of supporting data), others have abandoned HCC surveillance from frustration about the poor accuracy, leading to underuse of HCC screening in clinical practice [16]. Given the importance of early tumor detection for improving survival among HCC patients, there is a need for surveillance tests with higher sensitivity and specificity.

Biomarkers were put into practice to enhance earlier detection through less invasive means. AFP is a widely used and clinically approved biomarker for detection of hepatocellular carcinoma, with changes in the glycosylation site increasing its power to indicate a cancerous state [17–20]. AFP measurements can be taken directly from serum, allowing for a less invasive and more cost-effective screening method [21–24]. Combining AFP detection with US screening increased screening sensitivity to 90.2%, making this combination the most preferred method for the detection of hepatocellular carcinoma [25]. Recent reports have indicated that algorithms consisting of several clinical factors and patient information can be used to improve the performance of AFP [26, 27]. It is also noted that AFP may be associated with very specific types of HCC [28].

As with many cancers, outcome is greatly improved by early detection [29, 30]. Overall survival of those detected with early cancers is >60 months but <20 months if the cancer is caught at a later stage [31]. Hence there is great significance in the development of methods for the early detection of HCC.

Glycosylation and Biomarkers of Hepatocellular Carcinoma

Glycosylation, or the covalent addition of a carbohydrate chain to a protein, occurs through site-specific and enzyme-directed modification post- or co-translationally [32–34]. Glycosylation can occur in two forms: N-linked and O-linked. N-linked involves attachment of the carbohydrate chain to an asparagine residue with a consensus sequence of N-X-S/T (where X can be any amino acid except proline) and O-linked is attached to a serine or threonine residue. This modification occurring on cell surface proteins is crucial for cell-cell adhesion, signaling, and other cellular processes [35], and because of this dynamic variability, it is often a target for investigation as many disease states alter glycosylation expression [9, 22, 36]. As stated above, many current biomarkers are glycoprotein biomarkers—such as AFP for hepatocellular carcinoma—with the glycosylation playing an important role in the detection of the disease. It has been shown that many different structural motifs of these carbohydrate chains, or glycans, are associated with a disease state, such as increased branching, sialylation, fucosylation, or polylactosamine additions [22, 37–39]. Specifically for HCC, the addition of a core fucose (α1,6 linkage) to the associated N-glycosylation site on AFP is indicative of the disease [18].

Unfortunately, the use of these glycoprotein biomarkers is limited due to the lack of specificity for the tumor region. While serum is hepatic in origin and a viable option for biomarker detection of the disease, the sensitivity to earlier cases of hepatocellular carcinoma are still lacking. To more effectively detect earlier cases of HCC with higher degrees of specificity and sensitivity, more site-directed tissue analysis is necessary.

Broad Tissue Analysis in Hepatocellular Carcinoma

Tissue analysis for HCC, while often less favorable than serum studies, covers a wide range of analyses. Multi-omic studies approach the topic of characterizing hepatocellular carcinoma, including analyses in genomics, proteomics, transcriptomics, glycoproteomics, glycomics, and metabolomics. While some studies have a broad focus and touch on multi-omic approaches, others focus primarily on one to further elucidate possible changes and therapeutic targets between the cancerous region, cirrhotic tissue, and normal tissue. In broad studies, the focus is often on building a network that links many aspects of the specific cancer to determine affected pathways. For example, Ressom et al. characterized 499 genes, 217 proteins, 296 glycoproteins, 41 N-glycans, and 48 metabolites that represented significant changes between HCC and cirrhotic tissue, enabling the creation of a network that identified the most dysregulated pathways [40]. These findings demonstrated that tRNA charging, epithelial adherin junction remodeling, ILK signaling, EIF2 signaling, and glycolysis are significant pathways in the formation and maintenance of HCC. While these broad-scale studies usually don't lead to therapeutic targets, they provide a starting point for more specific -omic studies to move forward. Recently, one of the largest studies involved a genomic characterization of tissue via multiple platforms [4]. These researchers were able to corroborate and expand on the previous findings, determining that genes altered more significant pathways such as β-catenin/WNT and RTK/RAS/PI(3)K and other factors such as mutations in *TERT*, *TP53*, and *CTNNB1* genes, and immune checkpoints [4, 28, 41]. These genomic studies of tissue are increasingly important in HCC as our knowledge of the tumor heterogeneity increases. It has been well studied that HCC displays frequent heterogeneous growth patterns and features, often within the same tumor, making it difficult to accurately determine a specific pathway or gene that fits precisely for each case [28, 42, 43]. With the successes of AFP as a viable serum biomarker, many studies have shifted to proteomic and glycomic studies of liver tissue in hopes of establishing a more encompassing method of detection or developing a therapeutic target.

HCC Tissue Proteomics

Tissue proteomics have long been studied in many disease states, with excised tissue being homogenized and digested for protein analysis and comparative studies against normal tissue samples. Because of the availability of serum and its hepatic

origin, many proteomic analyses have been done using serum, though tissue proteomics are of equal importance. Through these studies, links can be established between what is seen in serum and in tissue. Utilizing both top-down and bottom-up proteomics, researchers have been determining specific proteins associated with disease state, tissue morphology, and progression.

As stated previously, in tissue proteomics, AFP is a clinical biomarker that arose through proteomic research; however, others were also found to indicate HCC presence. Osteopontin, a biomarker that is measured in plasma, has also been studied in tissue, and an increase in both osteopontin and Bcl-2 has been found in surgically resected HCC patients, indicating a co-dependence between the two in the tumorigenesis of HCC [44–46]. Along with osteopontin, peroxiredoxin 3 (PRX3) was also identified as a marker for HCC, and it has shown an increase of expression on both the mRNA and protein levels in 94.9% of HCC cases [47]. In tissue analysis, PRX3 has been shown to indicate poor differentiation as associated with progression of the disease. Unfortunately, while these two markers have shown promising possibilities in the detection of HCC, they failed to detect HCC in the presence of high levels of cirrhosis, making them inappropriate for clinical application. With many proteins associated with HCC found to be glycosylated, glycomic studies have become more relevant—both in serum and liver tissue analysis—to better understand the role and function glycosylation plays in HCC progression and the viability of glycoproteins as a therapeutic agent.

HCC Glycomics

Glycomics has quickly become an emerging trend in the field of cancer biomarker development, and HCC is no exception [48–78]. In most cases, glycan analysis has been done with serum and not directly from the cancer tissue itself [51, 63–78].

Our work, and that of others, has documented significant alterations in serum N-linked glycosylation with the development of HCC [79–84]. Specifically, the alterations most often observed are increased levels of alpha-1,3- and alpha-1,6-linked fucosylation found on bi-, tri-, and tetra-antennary glycans and to a lesser extent alterations in high mannose and tetra-antennary glycans [51, 68, 70, 72, 78, 81, 83, 85–100]. We have examined many of these fucosylated proteins as biomarkers of HCC and are in the process of commercializing them. Our results have shown that no one marker is good enough to detect all HCC, but when these fucosylated glycoproteins are used as part of a diagnostic algorithm, AUROCs of >0.90 are obtained [100].

Prior work about the source cells of serum fucosylation has been performed by a number of groups (including us) with unclear conclusions. Initial work suggested that the genes involved in the production of GDP-fucose, the substrate for the fucosylation reaction, were increased in HCC as compared to surrounding normal tissue [101].

Support that transformed hepatocytes are the source cells for fucosylated proteins comes from our recent work where we showed that as hepatocytes dedifferentiate and undergo an EMT, they increase their level of fucosylation and upregulate

many of the genes involved in alpha-1,6-linked fucosylation [102]. This is consistent with studies in lung cancer, where the alpha-1,6-fucosyltransferase gene (FUT-8) was involved in EMT [103]. It has also been shown that in a mouse model, deletion of FUT8 inhibits chemical-induced HCC by the downregulation of cancer-associated signaling pathways [94, 104]. Importantly, while these recent studies highlight the importance of fucosylation in cancer development, they do not offer any true data on the source cell(s) for fucosylation in the human disease.

Although a simple question, the fundamental question of the source of increased serum fucosylation has remained unanswered. As stated, our group has previously performed glycan analysis on HCC tissue following homogenization and HPLC-based glycan analysis [105]. In that study, two surprising things were noticed. First, there was a much higher level of fucosylation observed in normal liver tissue as compared to human serum depleted of immunoglobulins (highly abundant non-liver-derived serum protein). And, second, while 8 out of 16 tissue pairs did have increased levels of fucosylation, statistically there was no change in fucosylated glycans when HCC tissue was compared to either normal liver tissue (from an independent liver or from distal untransformed tissue) [105]. However, that study had two major flaws. First, we did not have matching serum to allow for the analysis of both serum and tissue, so it could not be determined which of these patients had elevated fucose. Second, we have recently determined that the glycan profile of hepatocytes and other liver cells are substantially different. That is, while liver tissue from normal individuals contains high levels of fucosylation (Fig. 5.1a), purified human hepatocytes from the same individual have very little fucose (Fig. 5.1b). In contrast, another liver cell type, liver sinusoidal endothelial cells (LSEC), contains high levels of fucosylated glycan (Fig. 5.1c). This high level of fucosylation within LSEC can confound the results when tissue is homogenized and examined in a mixed population. That is, although an HCC tumor may be primarily composed of transformed hepatocytes, adjacent liver tissue used for comparison will contain a mix of cells. Thus, any comparison is not a “like for like” evaluation. This is true for glycan analysis, proteomic analysis, and expression data. We will address this for the first time using several orthogonal methods. In regard to glycan analysis of tissue, we propose a new method, MALDI imaging mass spectrometry (MALDI-IMS) [106]. This method bypasses the need for microdissection and solubilization of tissue prior to analysis [105]. When matrix is applied across the tissue section, desorption can be targeted to specific “points” in a pattern and the data rasterized. The resulting spectra can then be used to generate two-dimensional maps of hundreds of analytes directly from the surface of a tissue section. These molecular maps display the relative abundance and spatial distribution of these molecules. Thus, MALDI tissue profiling has the power to link the molecular detail of mass spectrometry with molecular histology, generating mass spectra correlated to locations within a thin tissue section [107–110].

Figure 5.2 presents an example of the type of data observed with MALDI-glycan imaging of HCC tissue. Figure 5.2a is the tissue following H&E staining, and the large tumor is clearly visible, surrounded by nonmalignant tissue. Figure 5.2b shows the distribution of one glycan, the tetra-antennary galactosylated branched glycan

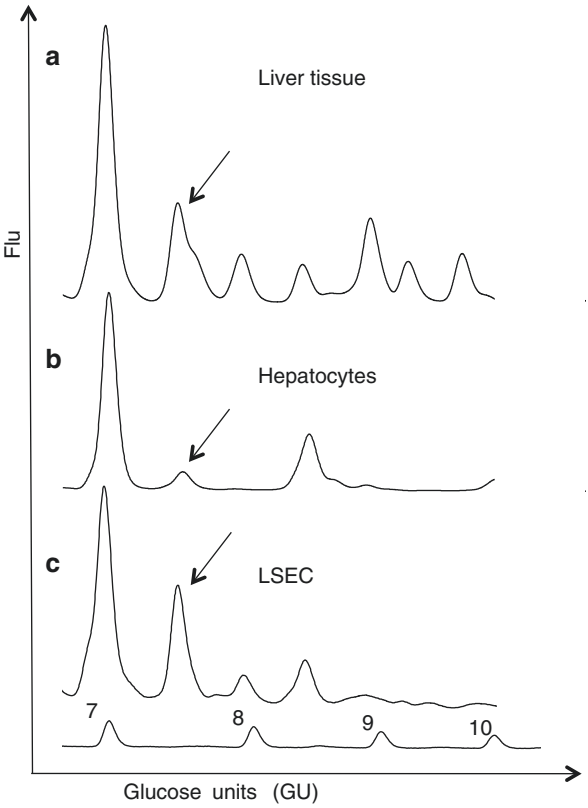

Fig. 5.1 (**a**) Desialylated N-linked glycan profile of liver tissue from a normal individual; (**b**) purified hepatocytes from that same individual; or (**c**) liver sinusoidal endothelial cells (LSEC) from that same individual. Arrow points to the bi-antennary fucosylated peak, the only fucosylated peak observed in hepatocytes. While liver tissue has high levels of fucosylation, as do LSEC, hepatocytes have low levels

(A4G4), in red. As this panel shows, the A4G4 glycan is found predominantly in the tumor region with little observed outside of the tumor. Similarly, the fucosylated version of this glycan (A4G4F1) was also found predominantly within the tumor region (Fig. 5.2c, in green). However, as Fig. 5.2d highlights, these glycans are differentially localized within the tumor, with the branched glycan without fucose predominantly in the inside of the tumor while the fucosylated branched glycan on the outside of the tumor. It is noted that in our recent study of 138 HCC tissue samples and 117 distally located adjacent nonmalignant tissue or cirrhotic tissue, 96% had increased levels of at least one fucosylated structure. Those patients were then categorized into the number of these highly branched and/or fucosylated structures they were presenting, with patients demonstrating increased levels in anywhere from one fucosylated structure all the way to 33 fucosylated structures [111].

As stated, one of the most observed cancer-associated glycosylation modifications is core fucosylation, though the exact reason is still unknown. Enzymatic activity was one of the first possibilities explored, as the increase of fucosyltransferase 8 (FUT8) through the β-catenin/WNT pathway is seen in many cancers including some HCC cases [103]. The use of core fucose-binding lectins for staining tissue has been used to determine the role of core fucosylation in HCC; however,

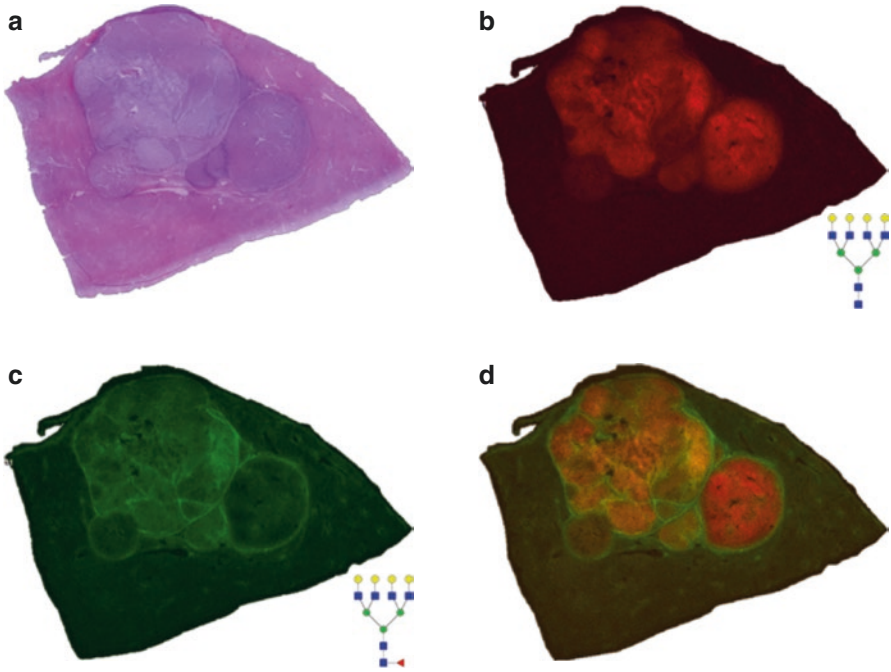

Fig. 5.2 MALDI-glycan imaging reveals regionally localized cancer-specific glycan signatures. (**a**) H&E stain of HCC tissue with surrounding nonmalignant tissue. (**b**) Localization of a glycan with a m/z value of 2393.840, which we have shown to be a tetra-antennary glycan. Red areas highlight localization of this glycan. (**c**) Localization of a glycan with a m/z value of 2539.957, which has been shown to be a tetra-antennary glycan with a single fucose residue. Green areas highlight areas of localization. (**d**) Overlay of Panels **b** and **c** which show distinct localization of these glycans within tissue

when comparing normal, cirrhotic, and HCC tissue staining, there does not appear to be a significant increase of fucosylation solely within HCC tissue but an overall trend within all tissue types [9, 112]. Along with core fucosylation, another glycan modification in HCC is increased glycan branching resulting in an increased presence of tetra-antennary glycans. These glycans are formed through β1,6 N-acetylglucosaminyltransferase V (MGAT-5) which results in an addition to tri-antennary glycans to form tetra-antennary structures [113]. This modification, more so than core fucosylation, has been seen in HCC tissue specifically and could play a role in the cancer's development and metastatic potential [111, 114, 115].

Future Directions and Conclusions

While tissue analysis of HCC has become more prevalent, there are still necessary steps required to link what is known regarding serum and tissue for more accurate biomarker discovery. Although biomarker discovery and analysis is moving in the

right direction and focusing more on the patient-specific tissue sections than simple circulating serum and plasma, most studies still fail to acknowledge the complex heterogeneity and morphology found within HCC tissue. In many cases, a tissue block or section is obtained and homogenized for analysis. This method disregards all pathological and histological complexities within the tissue, often including normal adjacent tissue or cirrhotic tissue in the analysis. This is where many tissue analyses are lacking, in that they could possibly include patterns and expressions that are not associated with the cancer. This leads to challenges in the development of robust biomarkers and contributes to their inability to detect earlier stages of liver cancer and disease. In the future, linking serum glycoproteomics to specific tissue glycomics within the cancerous region itself will become increasingly important. The utilization of MALDI MSI techniques provides a substantial basis for further analysis and helps determine protein and glycosylation changes in specific regions that can be correlated to disease states. In combining this technique with other -omic approaches, there is the possibility to develop more sensitive and specific biomarkers for enhanced detection of hepatocellular carcinoma.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86. https://doi.org/10.1002/ijc.29210.
2. Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y, Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2015;21(37):10573–83. https://doi.org/10.3748/wjg.v21.i37.10573.
3. Block TM, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. Oncogene. 2003;22(33):5093–107. https://doi.org/10.1038/sj.onc.1206557.
4. Cancer Genome Atlas Research Network. Electronic Address: wheeler@bcm.edu, Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;169(7):1327–41 e23. https://doi.org/10.1016/j.cell.2017.05.046.
5. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis. 2007;27(1):55–76. https://doi.org/10.1055/s-2006-960171.
6. Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005;25(2):181–200. https://doi.org/10.1055/s-2005-871198.
7. Petrick JL, Braunlin M, Laversanne M, Valery PC, Bray F, McGlynn KA. International trends in liver cancer incidence, overall and by histologic subtype, 1978–2007. Int J Cancer. 2016;139(7):1534–45. https://doi.org/10.1002/ijc.30211.
8. Ryerson AB, Eheman CR, Altekruse SF, Ward JW, Jemal A, Sherman RL, et al. Annual report to the nation on the status of cancer, 1975–2012, featuring the increasing incidence of liver cancer. Cancer. 2016;122(9):1312–37. https://doi.org/10.1002/cncr.29936.
9. Mehta A, Herrera H, Block T. Glycosylation and liver cancer. Adv Cancer Res. 2015;126:257–79. https://doi.org/10.1016/bs.acr.2014.11.005.
10. Pang TC, Lam VW. Surgical management of hepatocellular carcinoma. World J Hepatol. 2015;7(2):245–52. https://doi.org/10.4254/wjh.v7.i2.245.
11. Heimbach J, Kulik LM, Finn R, Sirlin CB, Abecassis M, Roberts LR, et al. Aasld guidelines for the treatment of hepatocellular carcinoma. Hepatology (Baltimore, Md.). 2017. https://doi.org/10.1002/hep.29086.

12. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. https://doi.org/10.1371/journal.pmed.1001624.
13. van Meer S, de Man RA, Coenraad MJ, Sprengers D, van Nieuwkerk KM, Klumpen HJ, et al. Surveillance for hepatocellular carcinoma is associated with increased survival: results from a large cohort in the Netherlands. J Hepatol. 2015;63(5):1156–63. https://doi.org/10.1016/j.jhep.2015.06.012.
14. Singal AG, Nehra M, Adams-Huet B, Yopp AC, Tiro JA, Marrero JA, et al. Detection of hepatocellular carcinoma at advanced stages among patients in the HALT-C trial: where did surveillance fail? Am J Gastroenterol. 2013;108(3):425–32. https://doi.org/10.1038/ajg.2012.449.
15. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, Parikh ND, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. Hepatology. 2017;65(4):1196–205. https://doi.org/10.1002/hep.28895.
16. Joshi K, Mendler M, Gish R, Loomba R, Kuo A, Patton H, et al. Hepatocellular carcinoma surveillance: a national survey of current practices in the USA. Dig Dis Sci. 2014;59(12):3073–7. https://doi.org/10.1007/s10620-014-3256-6.
17. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. Cancer Epidemiol Biomark Prev. 2014;23(1):144–53. https://doi.org/10.1158/1055-9965.EPI-13-0870.
18. Aoyagi Y. Molecular discrimination between alpha-fetoprotein from patients with hepatocellular-carcinoma and nonneoplastic liver-diseases by their carbohydrate structures (review). Int J Oncol. 1994;4(2):369–83.
19. Aoyagi Y. Carbohydrate-based measurements on alpha-fetoprotein in the early diagnosis of hepatocellular carcinoma. Glycoconj J. 1995;12(3):194–9.
20. Aoyagi Y, Isemura M, Suzuki Y, Sekine C, Soga K, Ozaki T, et al. Change in fucosylation of alpha-fetoprotein on malignant transformation of liver cells. Lancet. 1986;1(8474):210.
21. Block T, Mehta AS, London WT. Hepatocellular carcinoma of the liver. Cancer Biomark. 2010;9(1–6):375–83. https://doi.org/10.3233/CBM-2011-0165.
22. Mehta A, Block TM. Fucosylated glycoproteins as markers of liver disease. Dis Markers. 2008;25(4–5):259–65.
23. Comunale MA, Wang M, Hafner J, Krakover J, Rodemich L, Kopenhaver B, et al. Identification and development of fucosylated glycoproteins as biomarkers of primary hepatocellular carcinoma. J Proteome Res. 2009;8(2):595–602. https://doi.org/10.1021/pr800752c.
24. Steel LF, Mattu TS, Mehta A, Hebestreit H, Dwek R, Evans AA, et al. A proteomic approach for the discovery of early detection markers of hepatocellular carcinoma. Dis Markers. 2001;17(3):179–89.
25. Singal AG, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. Cancer Epidemiol Biomark Prev. 2012;21(5):793–9. https://doi.org/10.1158/1055-9965.EPI-11-1005.
26. Wang M, Devarajan K, Singal AG, Marrero JA, Dai J, Feng Z, et al. The Doylestown algorithm: a test to improve the performance of AFP in the detection of hepatocellular carcinoma. Cancer Prev Res (Phila). 2016;9(2):172–9. https://doi.org/10.1158/1940-6207.CAPR-15-0186.
27. Mehta AS, Lau DT, Wang M, Islam A, Nasir B, Javaid A, et al. Application of the Doylestown algorithm for the early detection of hepatocellular carcinoma. PLoS One. 2018;13(8):e0203149. https://doi.org/10.1371/journal.pone.0203149.
28. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res. 2009;69(18):7385–92. https://doi.org/10.1158/0008-5472.CAN-09-1089.
29. Marrero JA, Pelletier S. Hepatocellular carcinoma. Clin Liver Dis. 2006;10(2):339–51.

30. Lok A, McMahon B. Chronic hepatitis B. Hepatology (Baltimore, Md.). 2001;34(6):1225–41.
31. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362(9399):1907–17. https://doi.org/10.1016/S0140-6736(03)14964-1.
32. Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Glycosylation and the immune system. Science. 2001;291(5512):2370–6.
33. Van den Steen P, Rudd PM, Dwek RA, Opdenakker G. Concepts and principles of O-linked glycosylation. Crit Rev Biochem Mol Biol. 1998;33(3):151–208. https://doi.org/10.1080/10409239891204198.
34. Opdenakker G, Rudd PM, Ponting CP, Dwek RA. Concepts and principles of glycobiology. FASEB J. 1993;7(14):1330–7.
35. Christiansen MN, Chik J, Lee L, Anugraham M, Abrahams JL, Packer NH. Cell surface protein glycosylation in cancer. Proteomics. 2014;14(4–5):525–46. https://doi.org/10.1002/pmic.201300387.
36. Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta. 1999;1473(1):21–34.
37. Elola MT, Blidner AG, Ferragut F, Bracalente C, Rabinovich GA. Assembly, organization and regulation of cell-surface receptors by lectin-glycan complexes. Biochem J. 2015;469(1):1–16. https://doi.org/10.1042/BJ20150461.
38. Cheung P, Dennis JW. Mgat5 and Pten interact to regulate cell growth and polarity. Glycobiology. 2007;17(7):767–73. https://doi.org/10.1093/glycob/cwm037.
39. Takahashi M, Kizuka Y, Ohtsubo K, Gu J, Taniguchi N. Disease-associated glycans on cell surface proteins. Mol Asp Med. 2016;51:56–70. https://doi.org/10.1016/j.mam.2016.04.008.
40. Ressom HW, Di Poto C, Ferrarini A, Yunli H, Nezami Ranjbar MR, Ehwang S, et al. Multi-omic approaches for characterization of hepatocellular carcinoma. Conf Proc IEEE Eng Med Biol Soc. 2016;2016:3437–40. https://doi.org/10.1109/EMBC.2016.7591467.
41. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene. 2010;29(36):4989–5005. https://doi.org/10.1038/onc.2010.236.
42. Lu LC, Hsu CH, Hsu C, Cheng AL. Tumor heterogeneity in hepatocellular carcinoma: facing the challenges. Liver Cancer. 2016;5(2):128–38. https://doi.org/10.1159/000367754.
43. Friemel J, Rechsteiner M, Frick L, Bohm F, Struckmann K, Egger M, et al. Intratumor heterogeneity in hepatocellular carcinoma. Clin Cancer Res. 2015;21(8):1951–61. https://doi.org/10.1158/1078-0432.CCR-14-0122.
44. Kim J, Ki SS, Lee SD, Han CJ, Kim YC, Park SH, et al. Elevated plasma osteopontin levels in patients with hepatocellular carcinoma. Am J Gastroenterol. 2006;101(9):2051–9. https://doi.org/10.1111/j.1572-0241.2006.00679.x.
45. Deng B, Zhang XF, Zhu XC, Huang H, Jia HL, Ye QH, et al. Correlation and prognostic value of osteopontin and Bcl-2 in hepatocellular carcinoma patients after curative resection. Oncol Rep. 2013;30(6):2795–803. https://doi.org/10.3892/or.2013.2737.
46. Abu El Makarem MA, Abdel-Aleem A, Ali A, Saber R, Shatat M, Rahem DA, et al. Diagnostic significance of plasma osteopontin in hepatitis C virus-related hepatocellular carcinoma. Ann Hepatol. 2011;10(3):296–305.
47. Qiao B, Wang J, Xie J, Niu Y, Ye S, Wan Q, et al. Detection and identification of peroxiredoxin 3 as a biomarker in hepatocellular carcinoma by a proteomic approach. Int J Mol Med. 2012;29(5):832–40. https://doi.org/10.3892/ijmm.2012.916.
48. Nishimori I, Perini F, Mountjoy KP, Sanderson SD, Johnson N, Cerny RL, et al. N-acetylgalactosamine glycosylation of MUC1 tandem repeat peptides by pancreatic tumor cell extracts. Cancer Res. 1994;54(14):3738–44.
49. Hollingsworth MA, Strawhecker JM, Caffrey TC, Mack DR. Expression of MUC1, MUC2, MUC3 and MUC4 mucin mRNAs in human pancreatic and intestinal tumor cell lines. Int J Cancer. 1994;57(2):198–203.
50. Chambers JA, Hollingsworth MA, Trezise AE, Harris A. Developmental expression of mucin genes MUC1 and MUC2. J Cell Sci. 1994;107(Pt 2):413–24.

51. Liu Y, He J, Li C, Benitez R, Fu S, Marrero J, et al. Identification and confirmation of biomarkers using an integrated platform for quantitative analysis of glycoproteins and their glycosylations. J Proteome Res. 2010;9(2):798–805. https://doi.org/10.1021/pr900715p.
52. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer. 2004;4(1):45–60. https://doi.org/10.1038/nrc1251. nrc1251 [pii].
53. Batra SK, Kern HF, Worlock AJ, Metzgar RS, Hollingsworth MA. Transfection of the human Muc 1 mucin gene into a poorly differentiated human pancreatic tumor cell line, Panc1: integration, expression and ultrastructural changes. J Cell Sci. 1991;100(Pt 4):841–9.
54. Andrianifahanana M, Moniaux N, Schmied BM, Ringel J, Friess H, Hollingsworth MA, et al. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. Clin Cancer Res. 2001;7(12):4033–40.
55. Shibahara H, Tamada S, Higashi M, Goto M, Batra SK, Hollingsworth MA, et al. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. Hepatology (Baltimore, Md.). 2004;39(1):220–9. https://doi.org/10.1002/hep.20031.
56. Andrianifahanana M, Agrawal A, Singh AP, Moniaux N, van Seuningen I, Aubert JP, et al. Synergistic induction of the MUC4 mucin gene by interferon-gamma and retinoic acid in human pancreatic tumour cells involves a reprogramming of signalling pathways. Oncogene. 2005;24(40):6143–54. https://doi.org/10.1038/sj.onc.1208756. 1208756 [pii].
57. Singh AP, Chauhan SC, Bafna S, Johansson SL, Smith LM, Moniaux N, et al. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. Prostate. 2006;66(4):421–9. https://doi.org/10.1002/pros.20372.
58. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer. 2005;5(11):845–56. https://doi.org/10.1038/nrc1739. nrc1739 [pii].
59. Springer SA, Gagneux P. Glycomics: revealing the dynamic ecology and evolution of sugar molecules. J Proteome. 2016;135:90–100. https://doi.org/10.1016/j.jprot.2015.11.022.
60. Springer SA, Gagneux P. Glycan evolution in response to collaboration, conflict, and constraint. J Biol Chem. 2013;288(10):6904–11. https://doi.org/10.1074/jbc.R112.424523.
61. Dennis JW, Nabi IR, Demetriou M. Metabolism, cell surface organization, and disease. Cell. 2009;139(7):1229–41. https://doi.org/10.1016/j.cell.2009.12.008.
62. Goode G, Gunda V, Chaika NV, Purohit V, Yu F, Singh PK. MUC1 facilitates metabolomic reprogramming in triple-negative breast cancer. PLoS One. 2017;12(5):e0176820. https://doi.org/10.1371/journal.pone.0176820.
63. Lai KK, Kolippakkam D, Beretta L. Comprehensive and quantitative proteome profiling of the mouse liver and plasma. Hepatology (Baltimore, Md.). 2008;47(3):1043–51. https://doi.org/10.1002/hep.22123.
64. Mato JM, He F, Beretta L. The 2006 human liver proteome project (HLPP) workshops. Proteomics Clin Appl. 2007;1(5):442–5. https://doi.org/10.1002/prca.200700242.
65. Beretta L. Liver proteomics applied to translational research in liver disease and cancer. Proteomics Clin Appl. 2010;4(4):359–61. https://doi.org/10.1002/prca.201090012.
66. Yi X, Luk JM, Lee NP, Peng J, Leng X, Guan XY, et al. Association of mortalin (HSPA9) with liver cancer metastasis and prediction for early tumor recurrence. Mol Cell Proteomics. 2008;7(2):315–25. https://doi.org/10.1074/mcp.M700116-MCP200. M700116-MCP200 [pii].
67. Goldman R, Ressom HW, Varghese RS, Goldman L, Bascug G, Loffredo CA, et al. Detection of hepatocellular carcinoma using glycomic analysis. Clin Cancer Res. 2009;15(5):1808–13. https://doi.org/10.1158/1078-0432.CCR-07-5261. 1078-0432.CCR-07-5261 [pii].
68. Lattova E, McKenzie EJ, Gruwel ML, Spicer V, Goldman R, Perreault H. Mass spectrometric study of N-glycans from serum of woodchucks with liver cancer. Rapid Commun Mass Spectrom. 2009;23(18):2983–95. https://doi.org/10.1002/rcm.4202.
69. Isailovic D, Kurulugama RT, Plasencia MD, Stokes ST, Kyselova Z, Goldman R, et al. Profiling of human serum glycans associated with liver cancer and cirrhosis by IMS-MS. J Proteome Res. 2008;7(3):1109–17. https://doi.org/10.1021/pr700702r.

70. Amano M, Nishimura S. Large-scale glycomics for discovering cancer-associated N-glycans by integrating glycoblotting and mass spectrometry. Methods Enzymol. 2010;478:109–25. https://doi.org/10.1016/S0076-6879(10)78004-6. S0076-6879(10)78004-6 [pii].
71. Fang M, Dewaele S, Zhao YP, Starkel P, Vanhooren V, Chen YM, et al. Serum N-glycome biomarker for monitoring development of DENA-induced hepatocellular carcinoma in rat. Mol Cancer. 2010;9:215. https://doi.org/10.1186/1476-4598-9-215. 1476-4598-9-215 [pii].
72. Nakagawa T, Miyoshi E, Yakushijin T, Hiramatsu N, Igura T, Hayashi N, et al. Glycomic analysis of alpha-fetoprotein L3 in hepatoma cell lines and hepatocellular carcinoma patients. J Proteome Res. 2008;7(6):2222–33. https://doi.org/10.1021/pr700841q.
73. Nakagawa T, Takeishi S, Kameyama A, Yagi H, Yoshioka T, Moriwaki K, et al. Glycomic analyses of glycoproteins in bile and serum during rat hepatocarcinogenesis. J Proteome Res. 2010;9(10):4888–96. https://doi.org/10.1021/pr100414r.
74. An HJ, Lebrilla CB. A glycomics approach to the discovery of potential cancer biomarkers. Methods Mol Biol. 2010;600:199–213. https://doi.org/10.1007/978-1-60761-454-8_14.
75. An HJ, Kronewitter SR, de Leoz ML, Lebrilla CB. Glycomics and disease markers. Curr Opin Chem Biol. 2009;13(5–6):601–7. https://doi.org/10.1016/j.cbpa.2009.08.015. S1367-5931(09)00114-8 [pii].
76. Packer NH, von der Lieth CW, Aoki-Kinoshita KF, Lebrilla CB, Paulson JC, Raman R, et al. Frontiers in glycomics: bioinformatics and biomarkers in disease. An NIH white paper prepared from discussions by the focus groups at a workshop on the NIH campus, Bethesda MD (September 11–13, 2006). Proteomics. 2008;8(1):8–20. https://doi.org/10.1002/pmic.200700917.
77. Kirmiz C, Li B, An HJ, Clowers BH, Chew HK, Lam KS, et al. A serum glycomics approach to breast cancer biomarkers. Mol Cell Proteomics. 2007;6(1):43–55. https://doi.org/10.1074/mcp.M600171-MCP200. M600171-MCP200 [pii].
78. Zhu J, Lin Z, Wu J, Yin H, Dai J, Feng Z, et al. Analysis of serum haptoglobin fucosylation in hepatocellular carcinoma and liver cirrhosis of different etiologies. J Proteome Res. 2014;13(6):2986–97. https://doi.org/10.1021/pr500128t.
79. Chen R, Wang F, Tan Y, Sun Z, Song C, Ye M, et al. Development of a combined chemical and enzymatic approach for the mass spectrometric identification and quantification of aberrant N-glycosylation. J Proteome. 2012;75(5):1666–74. https://doi.org/10.1016/j.jprot.2011.12.015.
80. Zhao Y, Jia W, Wang J, Ying W, Zhang Y, Qian X. Fragmentation and site-specific quantification of core fucosylated glycoprotein by multiple reaction monitoring-mass spectrometry. Anal Chem. 2011;83(22):8802–9. https://doi.org/10.1021/ac201676a.
81. Ahn YH, Shin PM, Ji ES, Kim H, Yoo JS. A lectin-coupled, multiple reaction monitoring based quantitative analysis of human plasma glycoproteins by mass spectrometry. Anal Bioanal Chem. 2012;402(6):2101–12. https://doi.org/10.1007/s00216-011-5646-3.
82. Ahn YH, Shin PM, Oh NR, Park GW, Kim H, Yoo JS. A lectin-coupled, targeted proteomic mass spectrometry (MRM MS) platform for identification of multiple liver cancer biomarkers in human plasma. J Proteome. 2012;75:5507–15. https://doi.org/10.1016/j.jprot.2012.06.027.
83. Kamiyama T, Yokoo H, Furukawa J, Kurogochi M, Togashi T, Miura N, et al. Identification of novel serum biomarkers of hepatocellular carcinoma using glycomic analysis. Hepatology (Baltimore, Md.). 2013;57(6):2314–25. https://doi.org/10.1002/hep.26262.
84. Liu XE, Desmyter L, Gao CF, Laroy W, Dewaele S, Vanhooren V, et al. N-glycomic changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. Hepatology. 2007;46(5):1426–35. https://doi.org/10.1002/hep.21855.
85. Ajdukiewicz AB, Kelleher PC, Krawitt EL, Walters CJ, Mason PB, Koff RS, et al. Alpha-fetoprotein glycosylation is abnormal in some hepatocellular carcinoma, including white patients with a normal alpha-fetoprotein concentration. Cancer Lett. 1993;74(1–2):43–50.
86. Aoyagi Y, Suzuki Y, Igarashi K, Yokota T, Mori S, Suda T, et al. Highly enhanced fucosylation of alpha-fetoprotein in patients with germ cell tumor. Cancer. 1993;72(2):615–8.

87. Block TM, Comunale MA, Lowman M, Steel LF, Romano PR, Fimmel C, et al. Use of targeted glycoproteomics to identify serum glycoproteins that correlate with liver cancer in woodchucks and humans. Proc Natl Acad Sci U S A. 2005;102(3):779–84. https://doi.org/10.1073/pnas.0408928102.
88. Comunale MA, Lowman M, Long RE, Krakover J, Philip R, Seeholzer S, et al. Proteomic analysis of serum associated fucosylated glycoproteins in the development of primary hepatocellular carcinoma. J Proteome Res. 2006;5(2):308–15. https://doi.org/10.1021/pr050328x.
89. Comunale MA, Rodemich-Betesh L, Hafner J, Wang M, Norton P, Di Bisceglie AM, et al. Linkage specific fucosylation of alpha-1-antitrypsin in liver cirrhosis and cancer patients: implications for a biomarker of hepatocellular carcinoma. PLoS One. 2010;5(8):e12419. https://doi.org/10.1371/journal.pone.0012419.
90. Dai Z, Liu YK, Cui JF, Shen HL, Chen J, Sun RX, et al. Identification and analysis of altered alpha1,6-fucosylated glycoproteins associated with hepatocellular carcinoma metastasis. Proteomics. 2006;6(21):5857–67. https://doi.org/10.1002/pmic.200500707.
91. Dai Z, Zhou J, Qiu SJ, Liu YK, Fan J. Lectin-based glycoproteomics to explore and analyze hepatocellular carcinoma-related glycoprotein markers. Electrophoresis. 2009;30(17):2957–66. https://doi.org/10.1002/elps.200900064.
92. Debruyne EN, Vanderschaeghe D, Van Vlierberghe H, Vanhecke A, Callewaert N, Delanghe JR. Diagnostic value of the hemopexin N-glycan profile in hepatocellular carcinoma patients. Clin Chem. 2010;56(5):823–31. https://doi.org/10.1373/clinchem.2009.139295.
93. Kelleher PC, Walters CJ, Myhre BD, Tennant BC, Gerin JL, Cote PJ. Altered glycosylation of alpha-fetoprotein in hepadnavirus-induced hepatocellular carcinoma of the woodchuck. Cancer Lett. 1992;63(2):93–9.
94. Wang Y, Fukuda T, Isaji T, Lu J, Im S, Hang Q, et al. Loss of alpha1,6-fucosyltransferase inhibits chemical-induced hepatocellular carcinoma and tumorigenesis by down-regulating several cell signaling pathways. FASEB J. 2015;29(8):3217–27. https://doi.org/10.1096/fj.15-270710.
95. Yin H, Lin Z, Nie S, Wu J, Tan Z, Zhu J, et al. Mass-selected site-specific core-fucosylation of ceruloplasmin in alcohol-related hepatocellular carcinoma. J Proteome Res. 2014;13(6):2887–96. https://doi.org/10.1021/pr500043k.
96. Zhang Y, Zhu J, Yin H, Marrero J, Zhang XX, Lubman DM. ESI-LC-MS method for haptoglobin fucosylation analysis in hepatocellular carcinoma and liver cirrhosis. J Proteome Res. 2015;14(12):5388–95. https://doi.org/10.1021/acs.jproteome.5b00792.
97. Zhu J, Wang Y, Yu Y, Wang Z, Zhu T, Xu X, et al. Aberrant fucosylation of glycosphingolipids in human hepatocellular carcinoma tissues. Liver Int. 2014;34(1):147–60. https://doi.org/10.1111/liv.12265.
98. Zhu J, Wu J, Yin H, Marrero J, Lubman DM. Mass spectrometric N-glycan analysis of haptoglobin from patient serum samples using a 96-well plate format. J Proteome Res. 2015;14(11):4932–9. https://doi.org/10.1021/acs.jproteome.5b00662.
99. Comunale MA, Wang M, Anbarasan N, Betesh L, Karabudak A, Moritz E, et al. Total serum glycan analysis is superior to lectin-FLISA for the early detection of hepatocellular carcinoma. Proteomics Clin Appl. 2013;7:690–700. https://doi.org/10.1002/prca.201200125.
100. Wang M, Sanda M, Comunale MA, Herrera H, Swindell C, Kono Y, et al. Changes in the glycosylation of kininogen and the development of a kininogen-based algorithm for the early detection of HCC. Cancer Epidemiol Biomark Prev. 2017;26(5):795–803. https://doi.org/10.1158/1055-9965.EPI-16-0974.
101. Moriwaki K, Noda K, Nakagawa T, Asahi M, Yoshihara H, Taniguchi N, et al. A high expression of GDP-fucose transporter in hepatocellular carcinoma is a key factor for increases in fucosylation. Glycobiology. 2007;17(12):1311–20. https://doi.org/10.1093/glycob/cwm094.
102. Mehta A, Comunale MA, Rawat S, Casciano JC, Lamontagne J, Herrera H, et al. Intrinsic hepatocyte dedifferentiation is accompanied by upregulation of mesenchymal markers, pro-

tein sialylation and core alpha 1,6 linked fucosylation. Sci Rep. 2016;6:27965. https://doi.org/10.1038/srep27965.
103. Chen CY, Jan YH, Juan YH, Yang CJ, Huang MS, Yu CJ, et al. Fucosyltransferase 8 as a functional regulator of nonsmall cell lung cancer. Proc Natl Acad Sci U S A. 2013;110(2):630–5. https://doi.org/10.1073/pnas.1220425110.
104. Wang Y, Fukuda T, Isaji T, Lu J, Gu W, Lee HH, et al. Loss of alpha1,6-fucosyltransferase suppressed liver regeneration: implication of core fucose in the regulation of growth factor receptor-mediated cellular signaling. Sci Rep. 2015;5:8264. https://doi.org/10.1038/srep08264.
105. Mehta A, Norton P, Liang H, Comunale MA, Wang M, Rodemich-Betesh L, et al. Increased levels of tetra-antennary N-linked glycan but not core fucosylation are associated with hepatocellular carcinoma tissue. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive. Oncology. 2012;21(6):925–33. https://doi.org/10.1158/1055-9965.EPI-11-1183.
106. Chaurand P, Stoeckli M, Caprioli RM. Direct profiling of proteins in biological tissue sections by MALDI mass spectrometry. Anal Chem. 1999;71(23):5263–70.
107. Caldwell RL, Caprioli RM. Tissue profiling by mass spectrometry: a review of methodology and applications. Mol Cell Proteomics: MCP. 2005;4(4):394–401. https://doi.org/10.1074/mcp.R500006-MCP200.
108. Schwamborn K, Caprioli RM. MALDI imaging mass spectrometry--painting molecular pictures. Mol Oncol. 2010;4(6):529–38. https://doi.org/10.1016/j.molonc.2010.09.002.
109. Schwamborn K, Caprioli RM. Molecular imaging by mass spectrometry--looking beyond classical histology. Nat Rev Cancer. 2010;10(9):639–46. https://doi.org/10.1038/nrc2917.
110. Dai C, Cazares LH, Wang L, Chu Y, Wang SL, Troyer DA, et al. Using boronolectin in MALDI-MS imaging for the histological analysis of cancer tissue expressing the sialyl Lewis X antigen. Chem Commun. 2011;47(37):10338–40. https://doi.org/10.1039/c1cc11814e.
111. West CA, Wang M, Herrera H, Liang H, Black A, Angel PM, et al. N-linked glycan branching and fucosylation are increased directly in Hcc tissue as determined through in situ glycan imaging. J Proteome Res. 2018;17(10):3454–62. https://doi.org/10.1021/acs.jproteome.8b00323.
112. Romano PR, Mackay A, Vong M, DeSa J, Lamontagne A, Comunale MA, et al. Development of recombinant Aleuria aurantia lectins with altered binding specificities to fucosylated glycans. Biochem Biophys Res Commun. 2011;414(1):84–9. https://doi.org/10.1016/j.bbrc.2011.09.027.
113. Liu Y, Liu H, Liu W, Zhang W, An H, Xu J. beta1,6-N-acetylglucosaminyltransferase V predicts recurrence and survival of patients with clear-cell renal cell carcinoma after surgical resection. World J Urol. 2015;33(11):1791–9. https://doi.org/10.1007/s00345-014-1451-x.
114. Powers TW, Holst S, Wuhrer M, Mehta AS, Drake RR. Two-dimensional N-glycan distribution mapping of hepatocellular carcinoma tissues by MALDI-imaging mass spectrometry. Biomol Ther. 2015;5(4):2554–72. https://doi.org/10.3390/biom5042554.
115. Mehta A, Norton P, Liang H, Comunale MA, Wang M, Rodemich-Betesh L, et al. Increased levels of tetra-antennary N-linked glycan but not core fucosylation are associated with hepatocellular carcinoma tissue. Cancer Epidemiol Biomark Prev. 2012;21(6):925–33. https://doi.org/10.1158/1055-9965.EPI-11-1183.

Chapter 6
Molecular Subtypes and Genomic Signatures of Hepatocellular Carcinoma for Prognostication and Therapeutic Decision-Making

Sung Hwan Lee, Sun Young Yim, Jae-Jun Shim, and Ju-Seog Lee

EPCAM

Introduction

New technological developments have frequently preceded major advances in biomedical research and medicine [1]. For example, the development of fluorescent DNA sequencing technique made it possible to establish the large-scale high-throughput technology needed for the human genome sequencing. Also, polymerase chain reaction (PCR), fluorescent DNA sequencing, and other techniques have enabled the discovery of over 6000 Mendelian disease genes [2]. The advent of the DNA sequencing technologies has now made it possible to measure every alteration in human genome and expression of all coding and noncoding genes in different tissues under variety of conditions. This high-throughput technology has therefore afforded biomedical scientists a unique opportunity to integrate the descriptive characteristics (i.e., "phenotype") of a biological system under study with the genomic readout (i.e., mutations, copy number alteration, and RNA expression). The opportunity to contemplate the integrated view of biological systems has provoked a shift in biological sciences away from the classical reductionism to systems biology [1, 3, 4]. The systems approach to a disease is based on the hypothesis that disease processes perturb the regulatory network of genes and proteins in a way that differ from the respective normal counterpart. Consequently, by using multiparametric measurements, it may be possible to transform current

S. H. Lee · S. Y. Yim · J.-J. Shim · J.-S. Lee (✉)
Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: jlee@mdanderson.org

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_6

diagnostic and therapeutic approaches and enable a predictive and preventive personalized medicine [4].

The application of next-generation DNA sequencing technologies to characterize tumors at the gene level has had significantly impacted clinical oncology [5–7]. In particular, global gene expression analysis of various human tumors has resulted in the identification of gene expression patterns or signatures related to tumor classification, disease outcome, and response to therapy. The microarray and DNA/RNA sequencing technologies have also been used to investigate the mechanism of action of specific cancer therapeutics.

Clinical Staging of Hepatocellular Carcinoma

It is well established that cancer even in the same tissue is a very heterogeneous disease that differs widely in clinical outcomes and in response to therapy. It is now clear that this heterogeneity is due to different molecular defects that can induce similar tumor phenotypes. Although histopathological and biochemical markers constitute important tools for identifying groups of tumors that differ with respect to prognosis and responses to treatments, the genes and molecular pathways associated with these markers have not been comprehensively defined. Global gene expression analysis of human tumors has already revealed the identification of gene expression patterns or signatures related to tumor classification, prognosis, and response to therapy [8, 9].

The goal of all staging systems is to separate patients into groups with homogeneous prognosis, which then form the bases for the selection of most appropriate treatments. Much work has been devoted to establishing prognostic models for hepatocellular carcinoma (HCC) by using clinical information and pathological classification in order to provide information at diagnosis on both survival and treatment options [10–15]. Although much progress has been made, many issues still remain unresolved. For example, a staging system that reliably separates patients with early HCC as well as intermediate to advanced HCC into homogeneous groups with respect to prognosis does not exist. This is of particular importance because the natural course of early HCC is unknown and the natural progression of intermediate and advanced HCC are known to be quite heterogeneous [15]. This is especially troublesome since the accuracy of imaging techniques is rapidly evolving and affording detection of early HCC nodules [16, 17]. Although the pathological diagnosis of high-grade dysplastic nodules (DN) and early HCC is at present controversial, it is likely that many HCCs evolve from the DN [18]. However, prognostic predictions based on morphological characteristics of these early lesions are still tentative. Due to early surveillance program and improvement in imaging systems, early-stage and small HCCs at diagnosis dramatically increased [19]. Prognosis of early-stage HCC is not well understood, and conventional parameters such as number of tumors and size of tumors may not well account for response to treatment and prognosis. Thus, future classification will have to identify new more relevant vari-

ables that discriminate between patients with small early HCC without any vascular or extrahepatic extension.

Molecular Profiling of Hepatocellular Carcinoma

Numerous studies dealing with gene expression profiling of HCC have appeared during the last 15 years (Table 6.1). The molecular profiling of HCC presents challenges that are not commonly seen in other human tumors. This is primarily due

Table 6.1 Clinically relevant genomic subtypes of HCC

First author	Year	Primary endpoint	Number of genes	Experimental platform	Reference
Norio Iizuka	2003	Prediction of early intrahepatic recurrence of HCC after curative resection	12	Oligonucleotide microarray	[25]
Yukinori Kurokawa	2004	Prediction of early recurrence of HCC	20	PCR-based array	[26]
Qing-hai Ye	2003	Prediction of hepatitis B virus-positive metastatic HCC	153	cDNA microarray	[27]
Stephanie Roessler	2010	Prediction of tumor relapse in early-stage HCC Patients	161	NCI oligo set microarray	[28]
Hyun Goo Woo	2008	Prediction of HBV-related HCC	628	Affymetrix U133A 2.0 array	[29]
Beatriz Mínguez	2011	Prediction of microscopic vascular invasion in HCV-related HCC	35	Affymetrix HG-U133 plus 2 array	[32]
Jean–Charles Nault	2013	Prediction of HCC recurrence after curative resection	5	Affymetrix HG133A array	[34]
Ju-Seog Lee	2004	Classification of prognostic subclass and prediction of overall survival in HCC	406	Oligonucleotide microarray	[8]
Ju-Seog Lee	2004	Comparison of the molecular features of mouse and human HCCs	329	Oligonucleotide microarray	[42]
Ju-Seog Lee	2006	Prediction of the cellular origin of the tumor (hepatobalst vs. hepatocyte)	907	Oligonucleotide microarray	[9]
Sandrine Boyault	2007	Transcriptome classification of HCC and potential therapeutic target	16	Affymetrix HG133A array	[48]
Taro Yamashita	2008	Classification system defined by EpCAM and AFP to reveal HCC subtypes	29	cDNA and Oligo microarray	[46]

(continued)

Table 6.1 (continued)

First author	Year	Primary endpoint	Number of genes	Experimental platform	Reference
Taro Yamashita	2009	EpCAM-positive HCC as a tumor-initiating cells with stem/progenitor cell features	793	cDNA and Oligo microarray	[47]
Derek Y. Chiang,	2008	Molecular classification of HCC using copy number alteration and gene expression data	~1000	Affymetrix HG-U133 plus 2	[49]
Hoshida	2009	Molecular subclasses of human HCC	619	cDNA & Oligo microarray	[54]
Hyun Goo Woo	2010	Identification of a cholangiocarcinoma-like gene expression trait in HCC	581	Affymetrix U133A 2.0 array	[52]
Xin-Rong Yang	2010	Investigate the prognostic values of putative hepatic stem/progenitor cell in HCC	14	Real-time qRTePCR analysis	[36]
Soomi Kim	2012	Prediction of overall survival in HCC	65	Illumina microarray platform	[41]
TCGA	2017	Comprehensive and integrative genomic characterization of HCC	528	Illumina Hiseq	[56]
TCGA	2017	Characterization of the clinical, pathological, and molecular features of nonproliferative HCCs	550	Various microarray, Illumina Hiseq	[58]
Yujin Hoshida	2008	Genomewide expression profiling of HCC correlated with survival outcome	186	Illumina DASL	[62]
Lindsay Y King	2015	Genomic and clinical prognostic index for hepatitis C-related early-stage cirrhosis	186	Illumina DASL	[65]
Ji-Hoon Kim	2014	Development of genomic predictor for identifying late recurrence and its clinical implications	233	Illumina HumanHT-12	[66]

to the complex pathogenesis of this cancer [20, 21]. HCC arises most commonly in cirrhotic livers following infection with HBV or HCV. However, HCC can also occur under variety of other conditions such as hemochromatosis, excessive alcohol consumption, and nonalcoholic steatohepatitis. Each of these conditions represents complex and different constellations of chromosomal aberrations and genetic and epigenetic alterations as well as changed molecular pathways. Nevertheless, global gene expression profiling, because of its extraordinary power of resolution, may currently be the most appropriate technology platform to molecularly resolve the complex pathogenesis of HCC. These datasets represent an impressive progress in the use of gene expression profiling in elucidating the molecular pathogenesis of

HCC and hold the promise of improving the diagnostic and prognostic prediction for HCC patients. The dataset is also large enough to warrant a critical examination of reproducibility and validation of the molecular classification of HCC and the predictive expression "signatures" (or markers) generated by the global gene expression profiling of HCC.

For the analysis of cancer genomic data, two general methods have been applied to uncover molecular subtypes significantly associated clinical outcomes [22]. Supervised approach intends to find a set of variables such as expressed genes or mutation frequency from tumors on the basis of which one can reliably predict clinical outcomes such as survival, recurrence, response to treatments, or any class of interest in patients. Unsupervised approach intends to find either a completely novel subset (or cluster) of patients that are not recognized previously or to uncover similarity among group of patients that were considered as clinically different ones. The goal is to find more details about underlying biology of tumors that are clinically different and identify robust biomarkers that can reliably classify patients for better management.

Prognostic Subtypes of Hepatocellular Carcinoma Redefined by Supervised Approaches

HCC recurrence is a serious complication following resection of the primary tumor and happens in 50% of cases 3 years after the operation [23]. In 75% of the cases, this is due to intrahepatic metastasis, whereas the remaining 25% are due to de novo HCC [24]. The major histopathological features that predict HCC recurrence are vascular invasion, degree of differentiation of the tumor, and multinodularity [23].

Several studies have employed supervised approach to gene expression profiling data to address the issue of HCC recurrence following resection and intrahepatic metastasis.

Iizuka et al. investigated mRNA expression data from 33 HCC tumors as training set with use of early version of oligonucleotide microarrays with only 6000 genes [25]. The training set was used in a supervised learning manner to construct a predictor with 12 genes. The predictive performance of the system was then compared on a blinded set of samples from 27 newly enrolled patients. The system correctly predicted early intrahepatic recurrence or nonrecurrence in 25 (93%) of 27 samples in the blinded set and had a positive predictive value of 88% and a negative predictive value of 95%. This study was the first one that demonstrated the potential of prognostic values of genomic data from HCC tumors.

In another study, Kurokawa et al. addressed the issue of intrahepatic recurrence by analyzing gene expression using a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)-based array platform of 3072 genes in 100 HCC patients [26]. The authors selected 92 genes that demonstrated distinct expression patterns differing significantly between recurrence and recurrence-free cases. Using the 20

top-ranked genes (from the 92 selected), the predictor correctly predicted the early intrahepatic recurrence for 29 of 40 cases within the validation group, with the odds ratio of 6.8 (95% CI 1.7–27.5, $p = 0.01$). The 2-year recurrence rates in the patients with the good signature and those with the poor signature were 29.4% and 73.9%, respectively. The authors further showed (using multivariate Cox analysis) that the 20-gene molecular signature was an independent indicator for recurrence (hazard ratio 3.82, 95% CI 1.44–10.10, $p = 0.007$).

Ye et al. analyzed the expression profiles of 67 primary and metastatic HCC samples from 40 patients [27]. Using a supervised machine learning algorithm, the authors generated a 153-gene-expression signature that permitted classification of metastatic HCC patients and patient survival. The authors further showed that the gene-expression signature of primary HCCs with accompanying metastasis was very similar to that of their corresponding metastases, implying that genes favoring metastasis progression were initiated in the primary tumors. Furthermore, osteopontin, which was identified as a lead gene in the signature, was overexpressed in metastatic HCC and an osteopontin-specific antibody effectively blocked HCC cell invasion in vitro and inhibited pulmonary metastasis of HCC cells in nude mice. This metastatic gene signature was further redefined and validated in follow-up study [28]. In multivariate analyses including various clinical risk factors and clinical staging, the metastasis signature was an independent prognostic predictor, especially applicable to early recurrence, and a poor prognostic factor mainly associated with metastatic dissemination of HCC cells but not late recurrence, an outcome contributed mainly by high carcinogenic activities of diseased livers.

Woo et al. applied similar approach to identify genes whose expression is significantly associated with early recurrence after curative-intent treatment [29]. Authors selected 628 genes as classifiers from gene expression data from 65 HBV-associated HCC tumors by using univariate Cox proportional hazard model. Prognostic significance of the recurrence signature was validated in independent cohort of HCC patients. Gene network analysis with the recurrence signature revealed that SP1 transcription complex might be prominent common regulators of genes that differed in expression between high risk and low risk of early recurrence.

Vascular invasion is significantly associated with recurrence and poor clinical outcome after curative treatment of HCC such as resection and liver transplantation [30]. Meta-analysis with 1500 HCC patients further supported that the presence of vascular invasion is a critical factor for selection of patients for curative treatment in addition to size and number of tumors [31]. Minguez et al. developed 35-gene-based predictor that can identify HCC patients with vascular invasion [32]. Interestingly, high expression of CD24, an adhesion receptor of activated endothelial cells and platelets, was significantly associated with vascular invasion [33]. In contrast, many of metabolic genes such as *GLYAT*, *UGT2B15*, *CYP3A4*, and *ADH4* were under-expressed in the tumors with vascular invasion.

Nault et al. carried out stepwise analysis of gene expression data from HCC to identify prognostic gene set. By analyzing gene expression data from previous studies, they first selected 103 candidate genes for further selection with qRT-PCR experiments. By applying univariate Cox analysis and a stepwise forward selection

and backward elimination approach to expression data of 103 genes from training datasets, they identified a panel of five genes (*TAF9*, *RAMP3*, *HN1*, *KRT19*, and *RAN*) showing the strongest prognostic relevance and constructed five-gene scores for validation in independent cohort of HCC patients [34]. The five genes reflected different signaling pathways deregulated in poor prognostic tumors. *KRT19* is related to the stem cell and progenitor feature and known to be associated with poor prognosis of HCC [9]. Authors suggested that the five-gene score could be used for better selection of patients for liver transplantation, for example, by extending the Milan criteria to good prognostic tumors even if tumor size is more than 5 cm [35]. Yang et al. used similar approach with genes related to hepatic stem and progenitor cells to identify prognostic genes [36]. In this study, the expression and clinical significance of putative hepatic stem cell genes and tumor angiogenesis-related genes were investigated by real-time RT-PCR first and later by immunohistochemistry in three independent cohorts of patients with HCC.

Prognostic Subtypes of Hepatocellular Carcinoma Uncovered by Unsupervised or Semi-supervised Approaches

By applying unsupervised analysis of global gene expression data from human HCC, Lee et al. identified two distinctive subclasses that are highly associated with the survival of the patients: subtype A and B represent tumors with a poor and better prognosis, respectively [8]. A limited number (1016 gene features representing 947 unique genes) of genes were identified that both predicted the length of survival of the HCC patients and provided new molecular insights into the pathogenesis of HCC. Because application of a knowledge-based annotation of the 947 genes revealed that cell proliferation is the best characteristic of subtype A, it was named National Cancer Institute Proliferation (NCIP) signature. Subtype A also displayed higher expression of genes involved in ubiquitination and histone modification. It is well established that the ubiquitin system is frequently deregulated in cancers [37] and has been proposed as a possible predictive marker for recurrence of human HCC [38, 39]. The predictive power of the NCIP signature was validated in independent HCC datasets [9, 40, 41]. Cross-species comparison of the signature also revealed mouse models best mimicking human subtypes A and B [42].

The hepatic stem (HS) cell subtype of HCC was defined as gene expression patterns resembling those found in fetal hepatic stem cells [9]. Interestingly, HS subtype is a subset of previously recognized poor prognostic subtype A of NCIP. Gene network analysis of HS signature revealed that AP1 transcription factors such as *FOS*, *FOSL2*, and *JUNB* are highly activated in HS subtype. Shared gene expression patterns of the HS subtype and hepatic stem cells suggest that this subtype of HCC may arise from adult hepatic progenitor cells. Further support for this idea is supplied by the finding that expression of well-known markers of hepatic oval cells, such as *KRT7*, *KRT19*, and *VIM*, is found in the HS subtype of HCC [43].

Hepatic stem cell-like subtype of HCC was also uncovered by independent study. Epithelial cell adhesion molecule (EpCAM) was predominantly expressed in hepatic progenitor cells or hepatic stem cells [44, 45]. In attempt to find subset of HCC with stem cell characteristics, Yamashita et al. identified 70 EpCAM-coexpressed genes in EpCAM-positive HCC for construction of prediction model [46]. Prognostic significance of EpCAM-positive HCC subtype was validated in large independent cohorts. Based on the EpCAM signature, which may be related to different liver cell lineages, authors proposed the four subtypes of HCC: EpCAM-positive and AFP-positive HCC as hepatic stem cell-like HCC, EpCAM-positive and AFP-negative as bile duct epithelium-like HCC, EpCAM-negative and AFP-positive HCC as hepatocytic progenitor-like HCC, and EpCAM-negative and AFP-negative HCC as mature hepatocyte-like HCC. Markers for hepatic progenitor cells such as KRT19 and KIT are more abundantly expressed in hepatic stem cell-like HCC, whereas mature hepatocyte-specific genes such as *CYP3A4* are more abundantly expressed in mature hepatocyte-like HCC. Later study demonstrated that EpCAM-positive HCC is highly invasive and EpCAM is account for invasiveness of these cancer cells [47].

Another unsupervised approach revealed six genomic subtypes of HCC (G1–G6) [48]. Each subtype showed characteristic genetic alterations. The tumors in G1–G3 subtypes were associated with high chromosomal instability compared to the tumors in G4–G6 subtypes. Among the frequently mutated genes, *CTNNB1* mutations were enriched in the G5–G6 subtypes, while mutations in *TP53* genes were significantly associated with the G2–G3 subtypes. *PIK3CA* mutations were associated with the G2 subgroup. Hypermethylation on promoters of *CDH1* and *CDKN2A* were most frequently observed in the G5–G6 and G3 subtypes, respectively.

Integrative analysis of genomic copy number alteration with mRNA expression data from HCC tumors uncovered five genomic subtypes [49]. A subtype is a unique subclass of HCC characterized by polysomy of chromosome 7 and the concomitant overexpression of many genes in this chromosome. Intriguingly, these tumors lack gains of chromosome 8q, which are the second most frequent chromosomal alterations in hepatocellular carcinomas and include the known oncogenes *MYC*, *PTK2*, and *COPS5* [50, 51]. *CTNNB1*-activated subtype was enriched for gain-of-function mutations in *CTNNB1* (mostly located in exon 3). Interferon (IFN)-related subtype overexpressing several IFN-stimulated genes was associated with smaller tumor size.

Woo et al. carried out semi-supervised analysis with pooled gene expression data from HCC and cholangiocarcinoma. They discovered that the subset of HCC tumors is highly similar to cholangiocarcinoma and named them cholangiocarcinoma-like HCC (CLHCC) [52]. Tumors in CLHCC subtype are characterized by high expression of markers for hepatic progenitor cells such as *KRT19*, *EPCAM*, and *PROM1*. As expected, CLHCC subtype is significantly associated with poor prognosis, and it was validated in multiple independent cohorts of HCC patients. The CLHCC tumors are enriched with the proliferation, metastasis/adhesion, and development-related functions reflecting their aggressive phenotype. Biological

characteristics of CLHCC signature are also well overlapped with multiple embryonic stem cell signatures as well as hepatic stem cell signature [9, 53].

Meta-analysis of gene expression data from eight independent patient cohorts uncovered three HCC subtypes (S1, S2, and S3), each correlated with clinical parameters such as tumor size, extent of cellular differentiation, and serum AFP levels [54]. Of the three subtypes, S1 and S2 subtypes are associated with poor prognosis of HCC patients and S3 subtype is characterized by less aggressive features, including preserved hepatocyte function, smaller and more differentiated tumor, and better prognosis. S1 subtype is characterized by activation of TGF-β pathway and CLHCC gene signature [9, 52, 55], while S2 subtype is characterized by stem cell markers such as *EPCAM*, *AFP*, and *GPC3*, activation of *IGF2* pathway, and relative suppression of interferon target genes and hepatoblastoma-like gene signature [46, 47]. A vascular invasion gene signature [32] is more strongly associated with the S2 subtype. Interestingly, a subset of the S3 subtype HCC is characterized by gain-of-function mutations in *CTNNB1*. S2 subtype is further characterized by proliferation as well as *MYC* and *AKT* activation, and S3 was associated with hepatocyte differentiation.

Kim et al. carried out meta-analysis with two prognostic gene expression signatures to find limited number of genes whose expression is significantly associated with the prognosis of HCC patients [41]. Of 1016 NCIP genes and 628 recurrence genes from previous studies [8, 29], only 65 genes were shared by both gene lists. For easier translation of prognostic genome signatures to clinics, author generated recurrence-risk scores based on expression of 65 genes. The risk score was developed using Cox coefficient values of 65 genes in the training set, and its robustness was validated in test sets. The risk score was a highly significant predictor of overall survival and recurrence-free survival. In multivariate analysis, the risk score was a significant risk factor among clinical variables examined together. Interestingly, authors found that a high risk score was significantly associated with activation of *AKT* and *IGF1R*, whereas a high frequency of mutations of *CTNNB1* was significantly associated with a low risk score.

In recent analysis of HCC genome data from The Cancer Genome Atlas (TCGA) project, investigators found that HCC with *IDH1/2* mutations has very unique gene expression [56]. Interestingly, many HCC tumors without IDH mutations have IDH signature, and those with IDH signature (IDH-like subtype) showed significantly poor survival after treatment. When compared with other molecular subtypes of HCC, the IDH-like HCC exhibited the highest similarity to an HS [9]. These samples exhibited similarity to Hoshida's S2 subtype [54] and CLHCC subtype [52] and had high risk scores based on a gene expression of 65 genes [41].

By applying iCluster approach that integrates all of genomic data including somatic mutation, copy number alteration, mRNA expression, miRNA expression, and DNA methylation data [57], TCGA investigator uncovered three genomic subtypes: iC1, iC2, and iC3 [56]. iC1 subtype is characterized by clinical associations with younger age, Asian ethnicity, and female gender. These tumors exhibited features such as higher tumor grade and presence of macrovascular invasion. Molecular correlations with iC1 included a low frequency of *CDKN2A* silencing,

CTNNB1 mutation, and *TERT* promoter mutations accompanied with low *TERT* expression. This subclass was associated with overexpression of proliferation marker genes such as *MYBL2*, *PLK1*, and *MKI67*. iC2 and iC3 subtypes exhibited a high frequency of *CDKN2A* silencing, *TERT* promoter mutations, *CTNNB1* mutations, and *HNF1A* mutation. Correlation with clinical variables reveals association of iC2 subtype with low-grade tumors and less microvascular invasion. iC3 subtype is characterized by a higher degree of chromosomal instability with distinct 17p loss, high frequency of *TP53* mutation, and hypomethylation of multiple CpG sites. When compared with Hoshida's 3 genomic subtypes, iC1 is highly similar to Hoshida S2 subtype whereas iC3 is highly similar to Hoshida S3 subtype.

Recent meta-analysis with pooled HCC gene expression data revealed four subtypes of HCC that are well associated with liver zonation program: periportal (PP) subtype, perivenous (PV) subtype, extracellular matrix (ECM) subtype, and stem cell (STEM) subtype [58]. PV subtype is enriched for somatic mutations in *CTNNB1* and expresses many genes involved in liver zonation such as *GLUL*, *HAL*, and *VNN1*. Likewise, PP-type HCCs expressed a host of amino acid-degrading enzymes, such as *ARG1* and *GLS2*, which were major hubs in the periportal gene network in liver. STEM subtype is highly related to previously recognized HS subtype [9].

Nontumor Genomic Signatures

It has long been recognized that survival prediction of HCC patients is more challenging than with most other cancers. This is, in case of HBV and HCV, the consequence of the underlying viral-driven nonneoplastic disease, i.e., chronic hepatitis and cirrhosis that can inflict functional impairment on the liver that may affect the outcome of the HCC patients [59]. In HCC, two distinct types of recurrence are known. Early recurrence arises from primary cancer cells disseminating to the surrounding liver and is usually observed within the first 2 years after surgery. In contrast, late recurrence, which is typically observed more than 2 year after surgery, appears to be a result of chronic liver damage known as the "field effect" and produces de novo tumors that are independent of resected primary tumors [60]. The two types of recurrence have different clinical courses and probably appear in distinct biological contexts [61]. For better disease management, it is therefore important to uncover the biological characteristics of each type of recurrence and to develop distinct molecular prognostication systems that can identify patients at high risk for either type.

Hoshida et al. characterized gene expression data from nontumor surrounding tissues from HCC patients to uncover critical genes that might reflect field effect in liver leading to HCC development later [62]. By applying leave-one-out cross-validation procedure, authors identified 186 genes whose expression is significantly associated with survival of HCC patients. Prognostic significance of the signature was validated in large independent cohort of HCC. In particular, while the signature is not associated with early recurrence after surgery, it was significantly associated

with late recurrence. Genes upregulated in poor prognostic subtype include those related to interferon signaling, activation of NFκB, and TNFα signaling pathway. Interestingly, the downstream targets of IL6 were strongly associated with the signature, which is consistent with the finding that IL6 plays key roles in protecting mice from chemically induced HCC development [63]. The 186-gene signature was further validated in more relevant clinical setting. It was significantly correlated with long-term outcomes including HCC development of patients with hepatitis C-related early-stage cirrhosis [64, 65]. Therefore, the signature might be used to identify patients with cirrhosis in most need of surveillance and strategies to prevent the development of HCC.

Kim et al. identified gene set whose expression is significantly associated with hepatic injury and regeneration (HIR) in human liver [66]. When applied to gene expression data from nontumor surrounding tissues of HCC patients, HIR signature was significantly associated with late recurrence. In contrast, tumor-derived 65-gene recurrence score [41] was only associated with early recurrence. Gene network analysis revealed that STAT3 might be key upstream regulator of HIR signature. Activation of STAT3 in HCC patients with high risk for late recurrence was validated by immunostaining of surrounding liver tissues. The outcomes of analysis strongly suggested that early and late recurrences are clinically different entities with distinctive biological characteristics. Thus, separate rational treatment recommendations should be developed for better management of HCC patients. For example, patients at high risk of late recurrence may benefit from the use of JAK/STAT pathway inhibitors after surgical resection. Because current staging systems and biomarkers are limited in their ability to assess patients' risk of recurrence and their potential benefit from adjuvant therapy, two genomic predictors specific for early and late recurrence may represent tools that could help refine treatment decisions based on molecular characteristics.

Conclusion and Perspective

Comprehensive molecular and genomic analyses of large cohorts of HCC have now uncovered clinically relevant genomic subtypes, characteristic genomic alterations associated with subtypes, and genomic predictors of these subtypes. The results from analysis of genomic data have started to impact both clinical decision-making in oncology and advanced our understanding of cancer biology, as well as facilitated the development of more effective therapies.

While most of these findings are very encouraging, there are substantial gaps in translating genomic subtypes to clinics. While many of discovered genomic subtypes are clinically relevant, its clinical utility is hampered by discrepant results, which are probably due to difference in technological platforms, patient population, preparation and processing of samples, and classification algorithms. However, some of genomic subtypes were repeatedly discovered by independent studies. For example, HS subtype from NCI study is subset of poor prognostic subtype of NCIP

classification and highly similar to EpCAM-positive subtype, CLHCC subtype, and IDH-like HCC [56]. PV subtype from meta-analysis study is subset of Hoshida's S3 subtype and highly similar to CTNNB1 subtype from Barcelona group's study [49]. Albeit the similarity among independent classifications still remains superficial level, these similarities clearly suggest that it is possible to find consensus among different genomic classification methods that are clinically significant and biologically meaningful.

Key limitation of genomic subtype in HCC is that they do not provide clinically actionable information that is essential for personalized treatment of patients. Although sorafenib, a multi-kinase inhibitor [67], is approved for first-line treatment of advanced HCC patients more than 10 years ago [68, 69], there are no studies demonstrating association of genomic subtypes with treatment response to sorafenib yet. Likewise, many of targeted drugs approved for treatment of HCC patients lacks biomarkers reflecting genomic subtypes. Therefore, it will be important to collect tumors in prospective clinical trials to connect genomic subtypes and response to treatment.

Finally, another limitation is lack of actionable targets in subtypes. Many known drivers of HCC such as *CTNNB1*, *TERT*, *MYC*, and *YAP1* have been considered to be undruggable targets. Furthermore, key drivers or therapeutic targets are not fully discovered yet in some genomic subtypes. Therefore, it is important to establish preclinical models that faithfully recapitulate pathogenesis of subtypes. Animal models that recapitulate human's physiology and clinical setting have been crucial for understanding hepatocarcinogenesis and improving the treatment of HCC. The perfect animal model should reproduce natural history, etiology, and pathology of human HCC that would allow not only to uncover molecular mechanisms of HCC development over time but also to examine and evaluate potential novel therapeutic approaches in preclinical setting.

References

1. Sander C. Genomic medicine and the future of health care. Science. 2000;287(5460):1977–8.
2. Stenson PD, Mort M, Ball EV, Evans K, Hayden M, Heywood S, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited. Hum Genet. 2017;136(6):665–77.
3. Guttmacher AE, Collins FS. Realizing the promise of genomics in biomedical research. JAMA. 2005;294(11):1399–402.
4. Tian Q, Price ND, Hood L. Systems cancer medicine: towards realization of predictive, preventive. J Intern Med. 2012;271(2):111–21.
5. Dienstmann R, Rodon J, Barretina J, Tabernero J. Genomic medicine frontier in human solid tumors: prospects and challenges. J Clin Oncol. 2013;31(15):1874–84.
6. Macconaill LE, Garraway LA. Clinical implications of the cancer genome. J Clin Oncol. 2010;28(35):5219–28.
7. Mendelsohn J. Personalizing oncology: perspectives and prospects. J Clin Oncol. 2013;31(15):1904–11.

8. Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology. 2004;40(3):667–76. https://doi.org/10.1002/hep.20375.
9. Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med. 2006;12(4):410–6. https://doi.org/10.1038/nm1377.
10. Calvet X, Bruix J, Gines P, Bru C, Sole M, Vilana R, Rodes J, et al. Prognostic factors of hepatocellular carcinoma in the west: a multivariate. Hepatology. 1990;12(4 Pt 1):753–60.
11. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with. J Hepatol. 1999;31(1):133–41.
12. Investigator C. A new prognostic system for hepatocellular carcinoma: a retrospective study of. Hepatology. 1998;28(3):751–5.
13. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, et al. Natural history of hepatocellular carcinoma and prognosis in relation to. Cancer. 1985;56(4):918–28.
14. Tan CK, Law NM, Ng HS, Machin D. Simple clinical prognostic model for hepatocellular carcinoma in developing. J Clin Oncol. 2003;21(12):2294–8.
15. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology. 2018;68(2):723–50. https://doi.org/10.1002/hep.29913.
16. Kim CK, Lim J, Lee WJ, Lee WJ. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic liver. J Ultrasound Med. 2001;20(2):99–104.
17. Lencioni R, Cioni D, Bartolozzi C. Tissue harmonic and contrast-specific imaging: back to gray scale in ultrasound. Eur Radiol. 2002;12(1):151–65.
18. Kojiro M, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. Semin Liver Dis. 2005;25(2):133–42.
19. Leykum LK, El-Serag HB, Cornell J, Papadopoulos KP. Screening for hepatocellular carcinoma among veterans with hepatitis C on disease. Clin Gastroenterol Hepatol. 2007;5(4):508–12.
20. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet. 2002;31(4):339–46. https://doi.org/10.1038/ng0802-339.
21. Marquardt JU, Andersen JB, Thorgeirsson SS. Functional and genetic deconstruction of the cellular origin in liver cancer. Nat Rev Cancer. 2015;15(11):653–67 LID. https://doi.org/10.1038/nrc4017.
22. Libbrecht MW, Noble WS. Machine learning applications in genetics and genomics. Nat Rev Genet. 2015;16(6):321–32. https://doi.org/10.1038/nrg3920.
23. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012;379(9822):1245–55.
24. Cheung ST, Chen X, Guan XY, Wong SY, Tai LS, Ng IO, So S, et al. Identify metastasis-associated genes in hepatocellular carcinoma through. Cancer Res. 2002;62(16):4711–21.
25. Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, et al. Oligonucleotide microarray for prediction of early intrahepatic recurrence of. Lancet. 2003;361(9361):923–9.
26. Kurokawa Y, Matoba R, Takemasa I, Nagano H, Dono K, Nakamori S, Umeshita K, et al. Molecular-based prediction of early recurrence in hepatocellular carcinoma. J Hepatol. 2004;41(2):284–91.
27. Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, et al. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using. Nat Med. 2003;9(4):416–23.
28. Roessler S, Jia HL, Budhu A, Forgues M, Ye Q-H, Lee J-S, Thorgeirsson SS, et al. A unique metastasis gene signature enables prediction of tumor relapse in. Cancer Res. 2010;70(24):10202–12.
29. Woo HG, Park ES, Cheon JH, Kim JH, Lee J-S, Park BJ, Kim W, et al. Gene expression-based recurrence prediction of hepatitis B virus-related human. Clin Cancer Res. 2008;14(7):2056–64.
30. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100(10):698–711.

31. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, et al. Predicting survival after liver transplantation in patients with hepatocellular. Lancet Oncol. 2009;10(1):35–43.
32. Minguez B, Hoshida Y, Villanueva A, Toffanin S, Cabellos L, Thung S, Mandeli J, et al. Gene-expression signature of vascular invasion in hepatocellular carcinoma. J Hepatol. 2011;55(6):1325–31.
33. Sagiv E, Memeo L, Karin A, Kazanov D, Jacob-Hirsch J, Mansukhani M, Rechavi G, et al. CD24 is a new oncogene, early at the multistep process of colorectal cancer. Gastroenterology. 2006;131(2):630–9.
34. Nault JC, De Reynies A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, Decaens T, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients. Gastroenterology. 2013;145(1):176–87.
35. Clavien PA, Lesurtel M, Bossuyt PMM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an. Lancet Oncol. 2012;13(1):e11–22 LID. https://doi.org/10.1016/S470-2045(11)70175-9.
36. Yang XR, Xu Y, Yu B, Zhou J, Qiu S-J, Shi G-M, Zhang B-H, et al. High expression levels of putative hepatic stem/progenitor cell biomarkers. Gut. 2010;59(7):953–62.
37. Pagano M, Benmaamar R. When protein destruction runs amok, malignancy is on the loose. Cancer Cell. 2003;4(4):251–6.
38. Shirahashi H, Sakaida I, Terai S, Hironaka K, Kusano N, Okita K. Ubiquitin is a possible new predictive marker for the recurrence of human. Liver. 2002;22(5):413–8.
39. Tu K, Yang W, Li C, Zheng X, Lu Z, Guo C, Yao Y, et al. Fbxw7 is an independent prognostic marker and induces apoptosis and growth arrest. Mol Cancer. 2014;13:110.
40. Kim JH, Sohn BH, Lee HS, Kim SB, Yoo JE, Park YY, et al. Genomic predictors for recurrence patterns of hepatocellular carcinoma: model derivation and validation. PLoS Med. 2014;11(12):e1001770. https://doi.org/10.1371/journal.pmed.1001770.
41. Kim SM, Leem SH, Chu IS, Park YY, Kim SC, Kim SB, et al. Sixty-five gene-based risk score classifier predicts overall survival in hepatocellular carcinoma. Hepatology. 2012;55(5):1443–52. https://doi.org/10.1002/hep.24813.
42. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet. 2004;36(12):1306–11. https://doi.org/10.1038/ng1481.
43. Roskams TA, Libbrecht L, Desmet VJ, Desmet VJ. Progenitor cells in diseased human liver. Semin Liver Dis. 2003;23(4):385–96.
44. Schmelzer E, Wauthier E, Reid LM, Reid LM. The phenotypes of pluripotent human hepatic progenitors. Stem Cells. 2006;24(8):1852–8.
45. Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao H, Moss N, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med. 2007;204(8):1973–87.
46. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of. Cancer Res. 2008;68(5):1451–61.
47. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology. 2009;136(3):1012–24. https://doi.org/10.1053/j.gastro.2008.12.004.
48. Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, Herault A, et al. Transcriptome classification of HCC is related to gene alterations and to new. Hepatology. 2007;45(1):42–52.
49. Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Res. 2008;68(16):6779–88.
50. Patil MA, Gutgemann I, Zhang J, Ho C, Cheung ST, Ginzinger D, Li R, et al. Array-based comparative genomic hybridization reveals recurrent chromosomal. Carcinogenesis. 2005;26(12):2050–7.
51. Okamoto H, Yasui K, Zhao C, Arii S, Inazawa J. PTK2 and EIF3S3 genes may be amplification targets at 8q23-q24 and are associated. Hepatology. 2003;38(5):1242–9.

52. Woo HG, Lee JH, Yoon JH, Kim CY, Lee HS, Jang JJ, Yi N-J, et al. Identification of a cholangiocarcinoma-like gene expression trait in. Cancer Res. 2010;70(8):3034–41.
53. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA, et al. An embryonic stem cell-like gene expression signature in poorly differentiated. Nat Genet. 2008;40(5):499–507.
54. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res. 2009;69(18):7385–92. https://doi.org/10.1158/0008-5472.CAN-09-1089.
55. Coulouarn C, Factor VM, Thorgeirsson SS. Transforming growth factor-beta gene expression signature in mouse hepatocytes. Hepatology. 2008;47(6):2059–67.
56. The Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;169(7):1327–41. https://doi.org/10.1016/j.cell.2017.05.046.
57. Shen R, Olshen AB, Ladanyi M. Integrative clustering of multiple genomic data types using a joint latent. Bioinformatics. 2009;25(22):2906–12.
58. Desert R, Rohart F, Canal F, Sicard MA-O, Desille M, Renaud S, et al. Human hepatocellular carcinomas with a periportal phenotype have the lowest. Hepatology. 2017;66(5):1502–18.
59. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected. Gastroenterology. 2004;126(4):1005–14.
60. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, et al. Risk factors contributing to early and late phase intrahepatic recurrence of. J Hepatol. 2003;38(2):200–7.
61. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence. Cancer. 2000;89(3):500–7.
62. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med. 2008;359(19):1995–2004.
63. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6. Science. 2007;317(5834):121–4.
64. Hoshida Y, Villanueva A, Sangiovanni A, Sole M, Hur C, Andersson K, Chung RT, et al. Prognostic gene expression signature for patients with hepatitis C-related. Gastroenterology. 2013;144(5):1024–30.
65. King LY, Canasto-Chibuque C, Johnson KB, Yip S, Chen X, Kojima K, et al. A genomic and clinical prognostic index for hepatitis C-related early-stage. Gut. 2015;64(8):1296–302.
66. Kim JH, Sohn BH, Lee HS, Kim SB, Yoo JE, Park YY, et al. Genomic predictors for recurrence patterns of hepatocellular carcinoma: model. PLoS Med. 2014;11(12):e1001770.
67. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating. Nat Rev Drug Discov. 2006;5(10):835–44.
68. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90. https://doi.org/10.1056/NEJMoa0708857.
69. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with. Lancet Oncol. 2009;10(1):25–34.

Chapter 7
Liquid Biopsy in Hepatocellular Carcinoma

Irun Bhan, Daniel A. Haber, Raymond T. Chung, and David T. Ting

Introduction

Liquid biopsy, the sampling of cellular material from a solid tumor that has actively or passively entered the bloodstream, is an exciting area of research in cancer diagnostics. Tumor-derived components amenable to liquid biopsy include circulating tumor cells, exosomes, and circulating nucleic acids such as cell-free DNA and noncoding RNA. While the detection of secreted proteins might technically fit the definition of liquid biopsy, the term is usually used to refer to newer techniques focusing on other cellular products or cells themselves. The concept of sampling a tumor through phlebotomy is inherently attractive due to the risks of sampling the primary tumor which, in the case of hepatocellular carcinoma, include tumor seeding along the biopsy tract, hemoperitoneum, pneumothorax, bile peritonitis and sampling error leading to false negative results. The risk of tumor seeding with tissue biopsy of suspected HCC is reported to be 2.7% [1]. In the setting of cirrhosis, major complications of liver biopsy occur in 1.5–2.6% of cases [2, 3]. Liquid biopsy also offers the patient the convenience of a blood draw over procedures with conscious sedation which require hours from preparation to recovery.

I. Bhan (✉)
Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA
e-mail: ibhan1@mgh.harvard.edu

D. A. Haber · D. T. Ting
Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA

R. T. Chung
Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_7

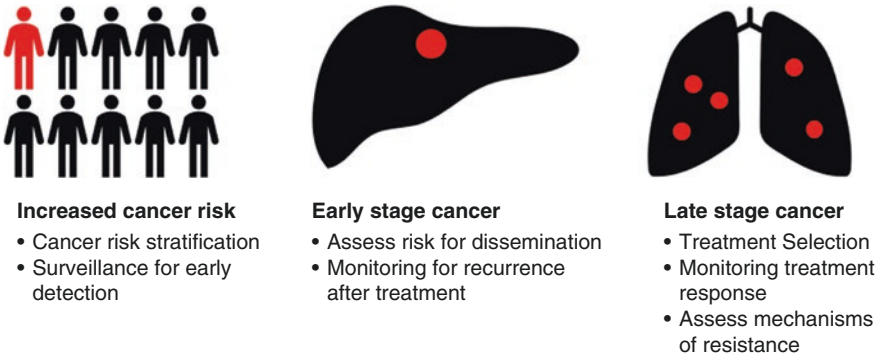

Fig. 7.1 The role of liquid biopsy according to disease stage in HCC. Blood-based biomarkers have the potential to improve each stage of cancer care

For these reasons, the application of liquid biopsy to biomarker development in hepatology and oncology has been an intense and rapidly expanding area of research in recent years.

Liquid biopsy development has focused on key areas of need in oncology that apply to each stage of cancer (Fig. 7.1). In individuals at high risk for developing HCC, such as those with cirrhosis or chronic hepatitis B infection, early detection of HCC through surveillance may facilitate curative treatment and improve long-term outcomes. However, commonly used surveillance tools including serum AFP and liver ultrasonography are suboptimal, suffering from low sensitivity for early lesions. Current areas of research include adapting liquid biopsy platforms to detect HCC lesions at an early, curable stage. For diagnosed early-stage cancer, liquid biopsy could play a role in risk stratification and detection of recurrence after treatment. In the setting of metastatic cancer, liquid biopsy could aid in treatment selection, monitoring of response, and understanding mechanisms of resistance. Each cellular component targeted by liquid biopsy has both strengths and weaknesses in addressing these clinical needs.

Circulating Tumor Cells

Background

Circulating tumor cells (CTCs) are cells shed from primary and metastatic sites of solid tumors into the bloodstream. CTCs were initially identified in 1869 during an autopsy of a woman with metastatic breast cancer [4]. Over 100 years later, the mechanism of entry of these cells into the vasculature is not well-understood but may involve both passive shedding of tumor cells facilitated by abnormal tissue vasculature and active migration of tumor cells as part of epithelial-to-mesenchymal transition. Upon entry into the circulation, many of these cells do not survive, but a

subset may carry additional functional gains required to persist including resistance to anoikis (apoptosis occurring when anchorage-dependent cells detach from the extracellular matrix) and evasion of the immune system [5]. The lifespan of these cells is likely several hours as most patients with localized cancer have no detectable CTCs at 24 hours after curative tumor resection [6]. Some CTCs gain the ability to intravasate into distant organs and coopt local tissues to create a supportive niche. Due to their varied mutational status, circulating tumor cells are heterogenous. The heterogeneity of CTCs is highlighted by comparisons of their morphology, proliferative index by Ki67 staining [6], and transcriptional profiling [7–9]. CTCs can circulate individually or in clusters. In the latter case, multicellular groupings are oligoclonal cells that may perform complementary metastatic functions [10].

Technology

The rarity of CTCs in the bloodstream creates challenges in the isolation of these cells. While a small number of patients may have high blood concentration of CTCs, even patients with metastatic cancer, who tend to have higher CTC concentrations, generally have fewer than 10 CTCs per mL of blood [11]. In a typical blood sample of a cancer patient, there may be one million-fold more white blood cells and one billion-fold more red blood cells than CTCs. CTC isolation technologies must balance achieving a high sensitivity for these rare and heterogenous cells while limiting contamination with white and red blood cells. CTC storage and processing must preserve the integrity of the cells and their informative cargo, including DNA, RNA, and protein. These technologies incorporate one or more of the following approaches to the isolation of CTCs: (1) size-based positive selection of CTCs, (2) positive selection of CTCs based on expected cell-surface marker expression, or (3) depletion of blood cells with collection of untagged CTCs (negative selection).

CTCs, consistent with their epithelial origin, tend to be larger than leukocytes (median diameter 15 μm vs. 10 μm) [12, 13]. Devices that filter CTCs based on size are attractive in their simplicity and ease of use. However, the hemodynamic forces required for filtration can cause cellular stress and damage, reducing cell viability and altering cell phenotype. Furthermore, due to heterogeneity in CTC size and the presence of large hematopoietic cells (such as bone marrow-derived megakaryocytes in patients undergoing chemotherapy), these devices could suffer from both reduced sensitivity and specificity [13–15]. Devices that include both size-based filtration and sorting based on additional physical properties such as deformability are under development and could provide superior results. Other physical properties that have been exploited for CTC isolation include photoacoustic resonance [16, 17], electrical charge [18], and differential density [19].

An alternative to a filtering-predominant approach is to detect the CTCs among the WBCs. One such platform involves plating CTCs and WBCs on an adherent surface, immunofluorescent staining of epithelial or tumor cell surface markers, and high-throughput microscopic scanning to identify CTCs [20]. Such an approach

facilitates enumeration of CTCs unbiased by cell size but may not allow for molecular analyses of the cells.

A popular approach involves the isolation and detection of CTCs using antibodies targeted against epithelial cell surface markers. This is the strategy employed by CellSearch (Menarini Silicon Biosystems), which, at the time of this writing, is the only platform for CTC assessment approved by the US Food and Drug Administration. In this assay, blood samples are fixed and CTCs are extracted in a magnetic field after being tagged with magnetic anti-EpCAM antibodies. Isolated cells are stained for DAPI to identify cell nuclei, additional epithelial markers (cytokeratins 8/18/19) to confirm identity of CTCs, and CD45 to highlight contaminating WBCs. Cells are imaged and candidate CTCs (DAPI-positive, cytokeratin-positive, CD45-negative) are displayed for final review by a human operator. The test is FDA-approved based on clinical studies demonstrating that CTCs identified by CellSearch are an independent predictor of overall and progression-free survival in metastatic breast [21], prostate [22], and colorectal cancer [23]. Limitations include an inability to interrogate cellular cargo (such as DNA or RNA) and reduced detection sensitivity due to cell loss through multiple processing steps and the requirement of EpCAM expression. Importantly, CTCs may lose expression of epithelial cell surface markers through epithelial-to-mesenchymal transition and may have particularly diminished or absent EpCAM expression in the setting of HCC, which is known to have low EpCAM expression [24, 25]. Other technologies use anti-EpCAM antibodies bound to various scaffolds to capture and eventually release CTCs but are liable to the same limitation of epithelial marker expression [26, 27].

Newer technologies rely on negative selection of CTCs through the depletion of white blood cells. The rationale is that WBCs have well-characterized cell surface markers that can be targeted for WBC removal, whereas targetable CTC antigens are incompletely understood. This strategy is employed by the CTC-iChip which is an integrated microfluidic device that removes red blood cells and platelets by size-based sorting and then deflects WBCs tagged with magnetic antibodies. CTCs, which remain untagged, are then collected for downstream bulk or single-cell analyses including enumeration, molecular characterization, and cell culture [15, 28]. Negative selection increases the sensitivity of CTC detection at the risk of contamination with WBCs that escape removal.

Applications in HCC

One of the first published works demonstrating the association of clinical outcomes and CTCs in HCC was by Matsumura et al. in 1999 who isolated peripheral blood nucleated cells by density centrifugation and identified CTCs among these cells by RT-PCR of alpha-fetoprotein mRNA as a marker of HCC origin [29]. The study followed 81 patients with biopsy-confirmed HCC confined to the liver who were undergoing locoregional therapy. The group found 64% of HCC patients tested positive for peripheral blood AFP mRNA which was associated with poorly

differentiated tumors and an increased incidence of extrahepatic metastasis. Patients with negative AFP mRNA after treatment demonstrated improved survival compared to patients with persistently positive levels. The utility of AFP mRNA as a prognostic marker was supported by subsequent studies [30, 31] but was also found to be positive in some patients with benign liver disease or cancers of non-hepatic origin [31, 32]. Other groups have studied various mRNA markers of HCC CTCs including MAGE 1, MAGE 3, GPC-3, CD44, and hTERT with variable success [33].

Studies employing the CellSearch system have generally demonstrated a sensitivity of 20–50% in identifying CTCs in HCC patients. Sun et al. enumerated CTCs using CellSearch in HCC patients undergoing potentially curative resection [34]. Using a threshold of 2 CTCs per 7.5 mL of peripheral blood, the group detected CTCs in 41% of patients preoperatively. CTC concentrations over this threshold were independently associated with postoperative tumor recurrence, even in subgroups thought to be otherwise at low risk for tumor recurrence such as those with Barcelona Clinic Liver Cancer stage 0 or A. The captured CTCs displayed stem cell-like phenotypes with expression of markers of cancer stem cell (CD133 and ABCG2), epithelial-mesenchymal transition, and Wnt pathway activation.

Emerging technologies have focused on depletion of white blood cells rather than positive selection of CTCs [15, 35, 36]. These technologies, which include the CTC-iChip, have the benefit of isolating unperturbed viable CTCs with high-quality RNA [7]. Our group combined the CTC-iChip with droplet digital PCR to create a CTC score based on the expression of liver-specific mRNA transcripts [28]. The CTC score had a sensitivity of 56% at a specificity of 95% for detecting HCC of any stage when using at-risk patients with chronic liver disease as controls. Notably, the CTC score decreased in HCC patients receiving therapy, suggesting a role for the platform in monitoring treatment response. Our follow-up study identified circulating cells of hepatic origin in patients with chronic liver disease (without hepatocellular carcinoma) using immunofluorescence and RNA sequencing after depletion of blood cells by the iChip [37]. These results suggest that liquid biopsy can detect preneoplastic "circulating epithelial cells." We created a machine learning algorithm based on RNA expression data to distinguish circulating epithelial cells of chronic liver disease and those of HCC (including early-stage disease), with an AUC of 0.927. The detection of circulating epithelial cells in preneoplastic disease has been noted in other solid organ disease including intraductal papillary mucinous neoplasm of pancreas [38–40]. The detection and analysis of these cells as they evolve from premalignant to malignant may facilitate using liquid biopsy for the early detection of cancer.

A large multicenter trial examined another negative enrichment strategy, RosetteSep Human CD45 Depletion Cocktail (STEMCELL Technologies), for CTC isolation in combination with RT-PCR of a panel of nine putative cancer stem cell mRNA transcripts for CTC detection in discovery, training, and validation cohorts (total of 1006 patients) [41]. In this study, Guo et al. narrowed the panel to four transcripts (EpCAM, CD90, CD133, CK19), which, when combined in a logistic regression model, demonstrated an AUC of 0.93 (sensitivity 82.1%, specificity 94.2%) in the validation cohort to differentiate HCC from a control

population of healthy blood donors and individuals with chronic hepatitis B, cirrhosis, or benign hepatic lesions. The platform's performance was similar in the detection of early-stage HCC and was superior to that of the standard serum biomarker AFP. Among patients undergoing resection for HCC, postoperative recurrence was associated with persistently positive CTC testing after surgery and higher preoperative CTC concentrations.

Studies to date of liquid biopsy in HCC have demonstrated the feasibility of detection and clinical relevance of CTCs. Research in HCC CTCs has been hampered by the use of multiple isolation and detection technologies, limiting the ability to compare results and draw definitive conclusions from the body of work. A meta-analysis of 20 studies with heterogenous methods calculated a pooled sensitivity of 67% at a specificity of 98% implying the included technologies would be inadequate to use alone for HCC diagnosis [42]. Whether CTCs can be useful as part of a multi-analyte surveillance regimen for the early diagnosis of HCC has not been adequately studied prospectively. On the other hand, enumeration of CTCs could play a role in monitoring response to therapy and evaluation of minimal residual disease given the demonstrated prognostic value of CTC testing. Studies successfully using molecular analysis of HCC CTCs raise hope that further research may lead to a better understanding of the mechanisms of metastasis and could allow for patient selection for emerging treatments such as immunotherapy. As new medical therapies for HCC develop, liquid biopsy could also facilitate personalized treatment through ex vivo culturing and treatment testing of CTCs.

Circulating Tumor DNA

Background

Cell-free DNA (cfDNA) are fragments of nucleic acids shed into the bloodstream from cells undergoing necrosis, apoptosis, or other forms of cell death [43]. ctDNA refers to the subset of cfDNA that is derived from tumors, whether from the primary site, metastatic deposits, or circulating tumor cells. cfDNA was first described in 1948 [44], but the identification of tumor-derived fragments was subsequently noted in the 1970s [45]. cfDNA typically circulates in fragments of approximately 180 bps which corresponds to the unit of chromatin protected by nucleosomes [46]. The plasma of healthy individuals typically carries less than 25 ng cfDNA per mL (the equivalent of several genomes) while in certain physiologic states, such as in the setting of inflammation or cancer, cfDNA concentrations are often several fold higher [45, 47]. Between cancer patients, the portion of cfDNA that is ctDNA is variable (ranging from <0.1% to >10%) which may reflect the underlying rate of cell turnover and cancer stage. However, within individuals, ctDNA fraction may track with tumor burden and response to treatment [48]. Tumor-specific genetic alterations, including point mutations, copy number changes, and gene rearrangements,

can be detected in ctDNA and reflect alterations found in the primary tumor in individual patients [49]. As ctDNA is shed from the entire tumor but tissue biopsies only sample a small portion, liquid biopsy of ctDNA may have an increased sensitivity for informative mutations which may only be present in a subpopulation of tumor cells. Liquid biopsy also allows for longitudinal sampling of an evolving cancer when repeat tissue biopsy carries prohibitive risk. These mutational analyses are helpful for cancers for which actionable mutations have been established. For example, specific ctDNA tests have been FDA-approved to detect BRAF V600E or V600K mutations (in melanoma to determine whether patients are candidates for targeted therapy), KRAS mutations (to identify colorectal cancer patients who may be ineligible for EGFR-targeted therapies), and EGFR mutations (to identify patients with non-small cell lung cancer who may benefit from targeted therapies). No specific tests have been approved to date for hepatocellular carcinoma, reflecting a lack of effective targeted therapies. Commercial tests for the detection of multiple tumor-associated mutations in ctDNA have also been approved. In such broad testing, a potential source of false positive results may be cfDNA derived from clonal expansions of nonmalignant cells that harbor typical tumor driver mutations. In blood cells, such nonmalignant proliferation has been termed "clonal hematopoiesis of indeterminate potential" as their mutations are associated with myeloid malignancy, but they generally do not progress to cancer [50]. A similar phenomenon has been described in solid organs [51, 52]. That cfDNA is a mixture of DNA derived throughout the body is both a strength and a weakness: it allows the sampling of all tumor sites (primary and metastatic as well as subpopulations therein) but makes identification of the tissue origin challenging. The analysis of DNA methylation patterns may allow for better tissue specificity [53].

Technologies

Compared to the isolation of circulating tumor cells, isolation of ctDNA is straightforward. Five to twenty milliliters of peripheral blood is drawn in a collection tube containing anticoagulant and preservatives. Cells are removed by centrifugation leaving plasma from which cfDNA can then be extracted using commercially available kits. cfDNA is generally procured from plasma rather than serum, due to the risk of increased non-tumor cfDNA from cell lysis when serum separator tubes are used. Distinguishing ctDNA from non-tumor cfDNA is a major challenge in liquid biopsy research, analogous to the "needle-in-a-haystack" problem of identifying CTCs among blood cells. Success depends on the sensitivity of the method employed for DNA analysis. While traditional RT-PCR has been used to identify point mutations, newer higher sensitivity methods with the potential for absolute quantification include digital PCR in which samples are diluted to one template per PCR reaction. Modifications of this approach include droplet digital PCR [54, 55] in which PCR reactions occur in separate droplets. Alternatively,

next-generation sequencing has become commonly used in the study of ctDNA for the detection of targeted mutations, whole exome sequencing or whole genome sequencing. Chromosomal rearrangements can also be detected by next-generation sequencing but requires the distinction of tumor-associated structural changes from germline copy number variants which can be facilitated by established bioinformatic filters [56]. While next-generation sequencing of ctDNA is performed clinically with FDA-approved tests, the concordance between assays has been called into question, a potential issue that will require further investigation [57]. An additional concern is that the amount of tumor-derived cfDNA varies as a function of total tumor burden. Most studies to date have focused on patients with advanced metastatic cancer, but in individuals with localized and potentially curable cancers, the ratio of signal to background in ctDNA-based mutation detection is less reliable.

Applications in HCC

Proof of principle for the detection of HCC point mutations in peripheral blood was demonstrated by Szymanska et al. who examined cfDNA for the presence of p53 R249S, a mutation described in patients with aflatoxin-associated HCC [58]. Plasma was collected from a longitudinal cohort monitored for the subsequent onset of HCC. Eight of fourteen patients carried the mutation in the tumor, and 9 carried R249S in cfDNA (as detected by short oligonucleotide mass analysis) with a concordance of 64%. Another study found a 22.2% concordance of HCC hotspot mutations in 27 matched resected tumor tissue specimens and plasma ctDNA samples [59]. The ctDNA samples were analyzed by digital droplet PCR which was noted to have a detection limit of 0.01%. Chan et al. demonstrated that copy number variations in four HCC patients could be detected in cfDNA prior to treatment but not after surgical tumor resection [60].

Cohen et al. developed a multi-analyte blood test for resectable cancer called CancerSEEK that incorporates ctDNA detection [61]. The platform determines a probability of cancer using plasma-based sequencing of 16 cancer-associated genes and the measurement of 8 cancer-associated serum proteins. A machine learning algorithm then combines these data with the measurement of a 31-protein panel to predict the tissue of origin of the cancer. The assay was developed to detect eight different solid cancers, including hepatocellular carcinoma. The initial portion of the test, which indicates the presence of any cancer, demonstrated a sensitivity of 100% for stage I hepatocellular carcinoma at a specificity of 99% when healthy individuals were used as the control population. In their cohort of eight cancer types, when cancer was detected in HCC patients, the machine learning algorithm was able to predict the tissue of origin with an accuracy of 44%. The assay is promising but will need to be evaluated in patients with chronic liver disease at high risk for HCC, the population who would most benefit from surveillance. It is possible the specificity may be reduced in this setting as the positive analytes could reflect the underlying liver disease rather than the HCC itself, a concern with any study that

relies on healthy control populations, rather than at-risk individuals who have cancer-predisposing conditions.

Several studies have examined the diagnostic potential of DNA methylation patterns which have been shown to be highly tissue-specific [62]. Kisiel et al. examined the accuracy of HCC detection using methylation patterns in cfDNA [53]. Candidate markers were identified by reduced representation bisulfite whole methylome sequencing on tissue DNA from HCC and control tissues. The candidate markers were evaluated in cfDNA from patients with HCC and cirrhotic controls. In the final assay, a six-marker panel (HOXA1, EMX1, AK055957, ECE1, PFKP, and CLEC11A) was scored using recursive partitioning decision analysis. In a cross-validated analysis of 95 HCC patients and 51 cirrhotic controls, the assay yielded a sensitivity of 95% at a specificity of 92%. The sensitivity for Barcelona Clinic Staging Criteria stage 0 was 75% and 93% for stage A. The selected genes were identified agnostic of biological significance but were subsequently noted to be involved in carcinogenesis.

Studies examining ctDNA in HCC have not yet explored the full technological power available in the field, which may partly be due to the lack of actionable mutations in HCC. There is, however, strong early data supporting the role of methylated ctDNA in the diagnosis of early HCC. Further validation is required.

Cell-Free Noncoding RNA

Background

Noncoding RNA (ncRNA) molecules are involved with various cellular processes including the regulation of gene expression. They are often categorized by their length as short and long species. The most studied variety of short noncoding is microRNAs (miRNAs) which are typically 21–25 nucleotides in length and regulate protein expression by binding mRNA at the 3'UTR, targeting the mRNA molecule for degradation or blocking translation. miRNAs have been shown to play roles in fundamental cell processes including differentiation, metabolism, and death. Long noncoding RNAs (lncRNAs) are >200 nucleotides in length and can modulate gene transcription through binding of regulatory proteins and complementary RNA or DNA. After cell lysis or from active secretion from cells, ncRNAs access the circulation as free nucleic acids or in membrane-bound extracellular vesicles [63].

Technology

The field of cell-free ncRNA is relatively new with the first description of circulating miRNA as a biomarker for solid tumors published in 2008 [64]. While extracellular RNA is susceptible to degradation by nucleases, miRNA is relatively resistant

to digestion compared with mRNA [65]. Studies examining circulating noncoding RNAs generally use commercially available kits for isolation of total RNA or miRNA from plasma followed by identification of RNA species by various methods including RT-PCR, digital PCR, microarray, NanoString (NanoString Technologies), or next-generation sequencing. While analysis of ctDNA focuses on the presence or absence of cancer-specific alterations, liquid biopsy of ncRNA relies on quantification of cancer-associated transcripts. Thus, ncRNA assessment requires either absolute transcript quantification or appropriate normalization to accurately compare levels between samples.

Applications in HCC

Numerous studies have examined the diagnostic capability of circulating miRNA in HCC. Okajima et al. identified candidate miRNAs with high expression in HCC tissue including miR-224 which was upregulated in HCC and HCC cell lines compared to normal tissues [66]. They found that miR-224 plasma levels detected by RT-PCR correlated with HCC tissue levels, decreased after resection of the primary tumor, correlated with tumor size and tumor recurrence, and was elevated in HCC patients compared to those with chronic liver disease without HCC. A meta-analysis suggested that panels of miRNAs may have better diagnostic characteristics than individual markers with miR-21, miR-199, and miR-122 providing improved specificity when using patients with liver disease as the control population [67]. Several studies have examined the utility of miRNA panels in HCC diagnosis, including a subsequently published work by Moshiri et al. [68]. Using RNA-seq, the group identified potential miRNA markers based on an initial analysis comparing plasma samples from patients with HCC, cirrhosis, and no known liver disease. They validated their findings in three small cohorts of HCC patients, cirrhotic patients, and healthy controls using droplet digital PCR to evaluate marker expression. A panel of three miRNA markers (miR-101-3p, miR-1246, and miR-106b-3p) scored by logistic regression performed with an AUC of 0.99 for distinguishing HCC and cirrhosis. The group demonstrated the feasibility of combining the discovery of miRNA markers of HCC by RNA-seq with subsequent validation using the higher throughput and more economical approach of droplet digital PCR.

The role of circulating lncRNA in liquid biopsy has only been studied in a limited fashion. Based on studies showing overexpression of the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in solid cancer including HCC [69], Konishi et al. investigated its role as a plasma biomarker of HCC [70]. While some HCC patients had elevated plasma MALAT-1 levels, many had levels similar to healthy controls or controls with chronic liver disease, limiting the sensitivity of using MALAT-1 alone as a diagnostic tool. Yan et al. performed quantitative RT-PCR for circulating lncRNAs reported to be associated with HCC

and found that a panel of three lncRNAs (LINC00152, XLOC014172, and RP11-160H22.5) and serum AFP in a logistic regression model was able to distinguish HCC patients from HBV chronic hepatitis patients with an AUC of 0.986 [71]. The ability of the panel to distinguish HCC patients and cirrhotic patients was not examined.

The detection of ncRNA as a liquid biopsy is a nascent field with numerous heterogenous studies suggesting different biomarkers may be useful in HCC diagnosis and prognosis. The relative ease of miRNA isolation, storage, and detection will facilitate a rapid accumulation of new data that will identify the most promising candidates.

Extracellular Vesicles

Background

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles that are released by both normal and diseased cells and carry various cellular molecules. They are categorized as apoptotic bodies, microvesicles (also called microparticles or ectosomes), and exosomes depending on their mechanism of generation. Apoptotic bodies are large cell fragments (usually with a diameter of over 500 nm) formed by blebbing during programmed cell death. Microvesicles are formed directly from blebbing of cell membranes and released into the extracellular space. Their diameter can range from 50 nm to 1000 nm, although in the setting of cancer, some larger microvesicles (termed "large oncosomes") have been observed. Exosomes are formed in a more complex manner from the endolysosomal pathway. First, the membrane of intracellular endosomes bulges inward and forms "intraluminal vesicles." The endosome subsequently fuses with the cell membrane, releasing the intraluminal vesicles as exosomes into the extracellular space. Exosomes are typically smaller than microvesicles, with a diameter of 30–100 nm. Biologically, EVs play a role in cell-to-cell communication, transferring various cellular molecules including proteins and nucleic acids to near or remote cells [72].

EVs have been reported to play an important role in carcinogenesis and metastasis. In a mouse model of pancreatic ductal adenocarcinoma, cancer-derived exosomes travel to the liver and induce changes in the hepatic microenvironment that facilitate liver metastases [73]. The role of EVs in hepatocarcinogenesis is less well studied. Wei et al. found that exosomes shuttle oncogenic miRNA between HCC cells, a process inhibited by the potential tumor suppressor Vps4a [74].

EVs have been detected in various body fluids, where they can be sampled for diagnostic means. The cargo can be interrogated to evaluate the cell of origin and query possible underlying disease states analogous to the analysis of circulating tumor cells.

Technology

As with circulating tumor cells, the technology for isolation of EVs is evolving. The associated techniques have not yet been standardized. Isolation techniques include differential ultracentrifugation, density gradient separation, immunoaffinity purification, and size-exclusion chromatography. EV research has largely used differential centrifugation with or without size-based exclusion by an additional filtration step, although the established protocols are lengthy and not conducive to high-throughput workflows for clinical care. Importantly, the particles isolated as well as the cargo detected can vary by technique [75]. While these factors have complicated the advancement and validation of research in EVs as biomarkers, several factors make them attractive candidates for ongoing study including the availability of newer commercial kits for EV isolation and the relative stability of these particles and their membrane-protected contents.

Applications in HCC

There has been hope that ncRNA isolated from EVs may show improved reproducibility as biomarkers over free circulating ncRNA due to the increased stability of nucleic acids in lipid-bound packaging where they are protected from endogenous nucleases [76]. Sohn et al. investigated the expression of several exosomal miRNAs in patients with HCC, cirrhosis, or chronic hepatitis B [77]. Exosomes were isolated from patient plasma using a kit-based system and the expression of ten miRNAs, selected based on published data on their expression in HCC tissue, was evaluated by RT-PCR. Levels of miR-18a, miR-221, and miR-222 were significantly elevated in HCC patients compared to patients with cirrhosis or chronic hepatitis but individually did not sufficiently discriminate between the patient populations for the purposes of diagnostic testing. Wang et al. examined miR-21 expression, which was previously shown to be elevated in solid tumors including HCC, using an exosome isolation kit and RT-PCR for quantification [78]. They found exosomal and free serum miR-21 expression were significantly elevated in HCC patients compared to those from chronic hepatitis B patients with overall higher expression and better discrimination using exosomal miRNA.

The possibility of using differentially expressed exosomal proteins as biomarkers was studied by Arbelaiz et al., who used mass spectrometry to examine the proteomes of exosomes derived from patients with primary sclerosing cholangitis, cholangiocarcinoma, or hepatocellular carcinoma [79]. Several potential exosomal protein biomarkers were identified including LG3BP which distinguished HCC patients and healthy controls with an AUC of 0.904 and FIBG which distinguished intrahepatic cholangiocarcinoma from HCC with an AUC of 0.894.

Given their mechanistic role in carcinogenesis, their multimolecular contents, and their stability, EVs have an exciting future in liquid biopsy; however, little is known about their role in HCC specifically at this time.

Conclusions

In recent years, there has been rapid development in blood-based analysis of solid tumors. The application of these technologies lags in HCC compared to other cancers, but as liver disease becomes a growing worldwide menace, we will see increasing applications to liver cancer. Advancement in the field will require standardization of isolation and analysis techniques and large-scale prospective studies with appropriate controls. The latter point regarding controls is especially important in the focus on early diagnosis: even screening tests with near-perfect sensitivity and specificity will suffer from poor positive predictive value if deployed in populations with low cancer incidence and prevalence. Thus, evaluating and employing diagnostic tests in high-risk populations (e.g., those with cirrhosis or chronic hepatitis B) is key. However, designing tests that distinguish HCC patients from chronic liver disease patients without HCC will likely be challenging. Many analytes that are elevated in HCC reflect an underlying proinflammatory or diseased state and may be elevated in nonmalignant liver disease as well. The role of liquid biopsy in HCC is currently strongest in cancer detection, monitoring, and prognostication but could evolve as novel therapies emerge that require identification of patient subgroups most likely to benefit. Technological issues that will continue to require attention include improvement in signal to noise due to the low concentration of tumor-derived components in the blood. As the sensitivity improves to detect subtle abnormalities, assessing cancer risk or detecting microscopic cancer and not just the presence of macroscopic cancer may be possible. The detection of invisible cancer will raise new questions in patient management for a disease that lacks effective chemoprevention or systemic therapy and for which curative treatment relies on the identification of radiographically detectable lesions.

Overall, each form of liquid biopsy has strengths and weaknesses in the noninvasive assessment of HCC. It is likely that not one but a combination of tests, perhaps both novel and conventional, will be required to improve detection and monitoring of HCC. The current body of work highlights exciting leads but is limited by heterogenous techniques, the need for further prospective validation, and the risk of false discovery associated with testing numerous markers simultaneously. Further work is still required before any of these technologies are ready for routine clinical application in HCC, but this is worthy of study: blood-based testing of a large and growing at-risk populations with liver disease could have major implications for the management of a deadly cancer.

References

1. Silva MA, Hegab B, Hyde C, Guo B, Buckels JAC, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. Gut. 2008;57(11):1592–6.
2. Maharaj B, Bhoora IG. Complications associated with percutaneous needle biopsy of the liver when one, two or three specimens are taken. Postgrad Med J. 1992;68(806):964–7.
3. Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol. 1986;2(2):165–73.
4. Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J. 1869;14:146.
5. Celià-Terrassa T, Kang Y. Distinctive properties of metastasis-initiating cells. Genes Dev. 2016;30(8):892–908.
6. Stott SL, Lee RJ, Nagrath S, Yu M, Miyamoto DT, Ulkus L, et al. Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. Sci Transl Med. 2010;2(25):25ra23–3.
7. Ting DT, Wittner BS, Ligorio M, Vincent Jordan N, Shah AM, Miyamoto DT, et al. Single-cell RNA sequencing identifies extracellular matrix gene expression by pancreatic circulating tumor cells. Cell Rep. 2014;8(6):1905–18.
8. Miyamoto DT, Lee RJ, Stott SL, Ting DT, Wittner BS, Ulman M, et al. Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. Cancer Discov. 2012;2(11):995–1003.
9. Heitzer E, Auer M, Gasch C, Pichler M, Ulz P, Hoffmann EM, et al. Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. Cancer Res. 2013;73(10):2965–75.
10. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014;158(5):1110–22.
11. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov. 2014;4(6):650–61.
12. Lin HK, Zheng S, Williams AJ, Balic M, Groshen S, Scher HI, et al. Portable filter-based microdevice for detection and characterization of circulating tumor cells. Clin Cancer Res. 2010;16(20):5011–8.
13. Mohamed H, Murray M, Turner JN, Caggana M. Isolation of tumor cells using size and deformation. J Chromatogr A. 2009;1216(47):8289–95.
14. Coumans FAW, van Dalum G, Beck M, Terstappen LWMM. Filter characteristics influencing circulating tumor cell enrichment from whole blood. Secomb TW, editor. PLoS One. 2013;8(4):e61770.
15. Ozkumur E, Shah AM, Ciciliano JC, Emmink BL, Miyamoto DT, Brachtel E, et al. Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. Sci Transl Med. 2013;5(179):179ra47–7.
16. Weight RM, Viator JA. Detection of circulating tumor cells by photoacoustic flowmetry. Methods Mol Biol. 2014;1102(Chapter 35):655–63.
17. Galanzha EI, Shashkov EV, Kelly T, Kim J-W, Yang L, Zharov VP. In vivo magnetic enrichment and multiplex photoacoustic detection of circulating tumour cells. Nat Nanotechnol. 2009;4(12):855–60.
18. Gascoyne PRC, Noshari J, Anderson TJ, Becker FF. Isolation of rare cells from cell mixtures by dielectrophoresis. Electrophoresis. 2009;30(8):1388–98.
19. Gertler R, Rosenberg R, Fuehrer K, Dahm M, Nekarda H, Siewert JR. Detection of circulating tumor cells in blood using an optimized density gradient centrifugation. Recent Results Cancer Res. 2003;162:149–55.

20. Krivacic RT, Ladanyi A, Curry DN, Hsieh HB, Kuhn P, Bergsrud DE, et al. A rare-cell detector for cancer. Proc Natl Acad Sci U S A. 2004;101(29):10501–4.
21. Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res. 2006;12(14 Pt 1):4218–24.
22. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302–9.
23. Cohen SJ, Punt CJA, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(19):3213–21.
24. de Boer CJ, van Krieken JH, Janssen-van Rhijn CM, Litvinov SV. Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver. J Pathol. 1999;188(2):201–6.
25. Went PT, Lugli A, Meier S, Bundi M, Mirlacher M, Sauter G, et al. Frequent EpCam protein expression in human carcinomas. Hum Pathol. 2004;35(1):122–8.
26. Talasaz AH, Powell AA, Huber DE, Berbee JG, Roh K-H, Yu W, et al. Isolating highly enriched populations of circulating epithelial cells and other rare cells from blood using a magnetic sweeper device. Proc Natl Acad Sci U S A. 2009;106(10):3970–5.
27. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007;450(7173):1235–9.
28. Kalinich M, Bhan I, Kwan TT, Miyamoto DT, Javaid S, LiCausi JA, et al. An RNA-based signature enables high specificity detection of circulating tumor cells in hepatocellular carcinoma. Proc Natl Acad Sci U S A. 2017;114(5):1123–8.
29. Matsumura M, Shiratori Y, Niwa Y, Tanaka T, Ogura K, Okudaira T, et al. Presence of alpha-fetoprotein mRNA in blood correlates with outcome in patients with hepatocellular carcinoma. J Hepatol. 1999;31(2):332–9.
30. Cillo U, Navaglia F, Vitale A, Molari A, Basso D, Bassanello M, et al. Clinical significance of alpha-fetoprotein mRNA in blood of patients with hepatocellular carcinoma. Clin Chim Acta. 2004;347(1–2):129–38.
31. Jeng K-S, Sheen I-S, Tsai Y-C. Does the presence of circulating hepatocellular carcinoma cells indicate a risk of recurrence after resection? Am J Gastroenterol. 2004;99(8):1503–9.
32. Lemoine A, Le Bricon T, Salvucci M, Azoulay D, Pham P, Raccuia J, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein mRNA in humans during liver surgery. Ann Surg. 1997;226(1):43–50.
33. Okajima W, Komatsu S, Ichikawa D, Miyamae M, Ohashi T, Imamura T, et al. Liquid biopsy in patients with hepatocellular carcinoma: circulating tumor cells and cell-free nucleic acids. World J Gastroenterol. 2017;23(31):5650–68.
34. Sun YF, Xu Y, Yang XR, Guo W, Zhang X, Qiu SJ, et al. Circulating stem cell–like epithelial cell adhesion molecule–positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. Hepatology. 2013;57(4):1458–68.
35. Guo W, Yang XR, Sun YF, Shen M-N, Ma X-L, Wu J, et al. Clinical significance of EpCAM mRNA-positive circulating tumor cells in hepatocellular carcinoma by an optimized negative enrichment and qRT-PCR-based platform. Clin Cancer Res. 2014;20(18):4794–805.
36. Sollier E, Go DE, Che J, Gossett DR, O'Byrne S, Weaver WM, et al. Size-selective collection of circulating tumor cells using Vortex technology. Lab Chip. 2014;14(1):63–77.
37. Bhan I, Mosesso K, Goyal L, Philipp J, Kalinich M, Franses JW, et al. Detection and analysis of circulating epithelial cells in liquid biopsies from patients with liver disease. Gastroenterology. 2018;155:2016–8.
38. Rhim AD, Thege FI, Santana SM, Lannin TB, Saha TN, Tsai S, et al. Detection of circulating pancreas epithelial cells in patients with pancreatic cystic lesions. Gastroenterology. 2014;146(3):647–51.

39. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. EMT and dissemination precede pancreatic tumor formation. Cell. 2012;148(1–2):349–61.
40. Franses JW, Basar O, Kadayifci A, Yuksel O, Choz M, Kulkarni AS, et al. Improved detection of circulating epithelial cells in patients with intraductal papillary mucinous neoplasms. Oncologist. 2018;23:121–7.
41. Guo W, Sun YF, Shen M-N, Ma X-L, Wu J, Zhang C-Y, et al. Circulating tumor cells with stem-like phenotypes for diagnosis, prognosis, and therapeutic response evaluation in hepatocellular carcinoma. Clin Cancer Res. 2018;24(9):2203–13.
42. Sun C, Liao W, Deng Z, Li E, Feng Q, Lei J, et al. The diagnostic value of assays for circulating tumor cells in hepatocellular carcinoma: a meta-analysis. Medicine (Baltimore). 2017;96(29):e7513.
43. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. Clin Chim Acta. 2001;313(1–2):139–42.
44. Mandel P, Seances CR. Les acides nucleiques du plasma sanguin chez l'homme. Soc Biol Fil. 1948;142:241–3.
45. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res. 1977;37(3):646–50.
46. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. Clin Chem. 2010;56(8):1279–86.
47. Perkins G, Yap TA, Pope L, Cassidy AM, Dukes JP, Riisnaes R, et al. Multi-purpose utility of circulating plasma DNA testing in patients with advanced cancers. Perez-Gracia JL, editor. PLoS One. 2012;7(11):e47020.
48. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985–90.
49. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58.
50. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
51. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science. 2015;348(6237):880–6.
52. Aghili L, Foo J, DeGregori J, De S. Patterns of somatically acquired amplifications and deletions in apparently normal tissues of ovarian cancer patients. Cell Rep. 2014;7(4):1310–9.
53. Kisiel JB, Dukek BA, Kanipakam RVSR, Ghoz HM, Yab TC, Berger CK, et al. Hepatocellular carcinoma detection by plasma methylated DNA: discovery, phase I pilot, and phase II clinical validation. Hepatology. 2019;69:1180–92.
54. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem. 2011;83(22):8604–10.
55. Pekin D, Skhiri Y, Baret J-C, Le Corre D, Mazutis L, Salem CB, et al. Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab Chip. 2011;11(13):2156–66.
56. Leary RJ, Sausen M, Kinde I, Papadopoulos N, Carpten JD, Craig D, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med. 2012;4(162):162ra154–4.
57. Torga G, Pienta KJ. Patient-paired sample congruence between 2 commercial liquid biopsy tests. JAMA Oncol. 2018;4(6):868–70.
58. Szymańska K, Chen J-G, Cui Y, Gong YY, Turner PC, Villar S, et al. TP53 R249S mutations, exposure to aflatoxin, and occurrence of hepatocellular carcinoma in a cohort of chronic hepatitis B virus carriers from Qidong, China. Cancer Epidemiol Biomark Prev. 2009;18(5):1638–43.
59. Huang A, Zhang X, Zhou S-L, Cao Y, Huang X-W, Fan J, et al. Detecting circulating tumor DNA in hepatocellular carcinoma patients using droplet digital PCR is feasible and reflects intratumoral heterogeneity. J Cancer. 2016;7(13):1907–14.

60. Chan KCA, Jiang P, Zheng YWL, Liao GJW, Sun H, Wong J, et al. Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem. 2013;59(1):211–24.
61. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926–30.
62. Aryee MJ, Wu Z, Ladd-Acosta C, Herb B, Feinberg AP, Yegnasubramanian S, et al. Accurate genome-scale percentage DNA methylation estimates from microarray data. Biostatistics. 2011;12(2):197–210.
63. Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;11(6):426–37.
64. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513–8.
65. Chim SSC, Shing TKF, Hung ECW, Leung T-Y, Lau T-K, Chiu RWK, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54(3):482–90.
66. Okajima W, Komatsu S, Ichikawa D, Miyamae M, Kawaguchi T, Hirajima S, et al. Circulating microRNA profiles in plasma: identification of miR-224 as a novel diagnostic biomarker in hepatocellular carcinoma independent of hepatic function. Oncotarget. 2016;7(33):53820–36.
67. Ding Y, Yan J-L, Fang A-N, Zhou W-F, Huang L. Circulating miRNAs as novel diagnostic biomarkers in hepatocellular carcinoma detection: a meta-analysis based on 24 articles. Oncotarget. 2017;8(39):66402–13.
68. Moshiri F, Salvi A, Gramantieri L, Sangiovanni A, Guerriero P, De Petro G, et al. Circulating miR-106b-3p, miR-101-3p and miR-1246 as diagnostic biomarkers of hepatocellular carcinoma. Oncotarget. 2018;9(20):15350–64.
69. Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene. 2007;26(6):851–8.
70. Konishi H, Ichikawa D, Yamamoto Y, Arita T, Shoda K, Hiramoto H, et al. Plasma level of metastasis-associated lung adenocarcinoma transcript 1 is associated with liver damage and predicts development of hepatocellular carcinoma. Cancer Sci. 2016;107(2):149–54.
71. Yuan W, Sun Y, Liu L, Zhou B, Wang S, Gu D. Circulating LncRNAs serve as diagnostic markers for hepatocellular carcinoma. Cell Physiol Biochem. 2017;44(1):125–32.
72. Hirsova P, Ibrahim SH, Verma VK, Morton LA, Shah VH, LaRusso NF, et al. Extracellular vesicles in liver pathobiology: small particles with big impact. Hepatology. 2016;64(6):2219–33.
73. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015;17(6):816–26.
74. Wei JX, Lv LH, le Wan Y, Cao Y, Li GL, Lin HM, et al. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. Hepatology. 2015;61(4):1284–94.
75. Witwer KW, Buzás EI, Bemis LT, Bora A, Lässer C, Lötvall J, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J Extracell Vesicles. 2013;2(1):20360.
76. Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles. 2014;3:23743.
77. Sohn W, Kim J, Kang SH, Yang SR, Cho J-Y, Cho HC, et al. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. Exp Mol Med. Nature Publishing Group. 2015;47(9):e184.
78. Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. Biomed Res Int. 2014;2014:864894.
79. Arbelaiz A, Azkargorta M, Krawczyk M, Santos-Laso A, Lapitz A, Perugorria MJ, et al. Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. Hepatology. 2017;66(4):1125–43.

Part III
Treatment

Chapter 8
Surgical Therapies in Hepatocellular Carcinoma

Caitlin A. Hester and Adam C. Yopp

Introduction

Hepatocellular cancer (HCC) is the seventh most common cancer and the fourth leading cause of cancer-related deaths worldwide [1]. It represents the fastest-growing cause of cancer-related deaths among males in the United States, and its incidence and mortality have increased threefold over the last 20 years. Among patients with cirrhosis, it represents the leading cause of death [2, 3]. HCC is inherently complex; its outcomes are not only associated with burden of disease but also underlying liver function. The challenge of management is in delivering curative treatment without precipitating further liver decompensation, and thus a multidisciplinary team is strongly associated with improved patient outcomes [4].

Curative treatment, defined as surgical resection, liver transplantation, and radiofrequency ablation (RFA), has been associated with a median overall survival (OS) of greater than 60 months, with 5-year survival rates approaching 70%. In clinical practice guidelines, curative surgical treatment is recommended for early-stage disease [5]. Recent implementation of HCC surveillance programs has resulted in earlier tumor diagnosis and thus created a greater demand for curative treatment among HCC patients [6, 7]. The aim of this chapter is to identify patients eligible to receive curative therapy, review the types and techniques of curative surgical therapies available in HCC management, namely resection and transplantation, and describe outcomes associated with various operative techniques.

C. A. Hester · A. C. Yopp (✉)
Division of Surgical Oncology, Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX, USA
e-mail: adam.yopp@utsouthwestern.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_8

Staging Systems to Determine Patient Eligibility for Curative Treatment

The decision to proceed with surgical resection or transplantation is multifaceted. Careful consideration of the patient's performance status, underlying liver function, and tumor biology including number of nodules, size of nodules, and vascular involvement is essential. A multidisciplinary approach including a medical oncologist, transplant hepatologist, transplant surgeon, surgical oncologist, radiation oncologist, interventional radiologist, and radiologist should be utilized for all patient management plans [4]. The multitude of published staging systems is evidence of the complexity of evaluating a newly diagnosed HCC patient. Various staging systems have been proposed, including American Joint Committee on Cancer tumor-node-metastasis (TNM), Okuda, and the Cancer of the Liver Italian Program (CLIP) staging systems [8–10]. According to the staging by American Association for the Study of Liver Diseases (AASLD), early-stage diseases are recommended to be treated by surgical therapies, including resection and transplantation (Fig. 8.1) [5].

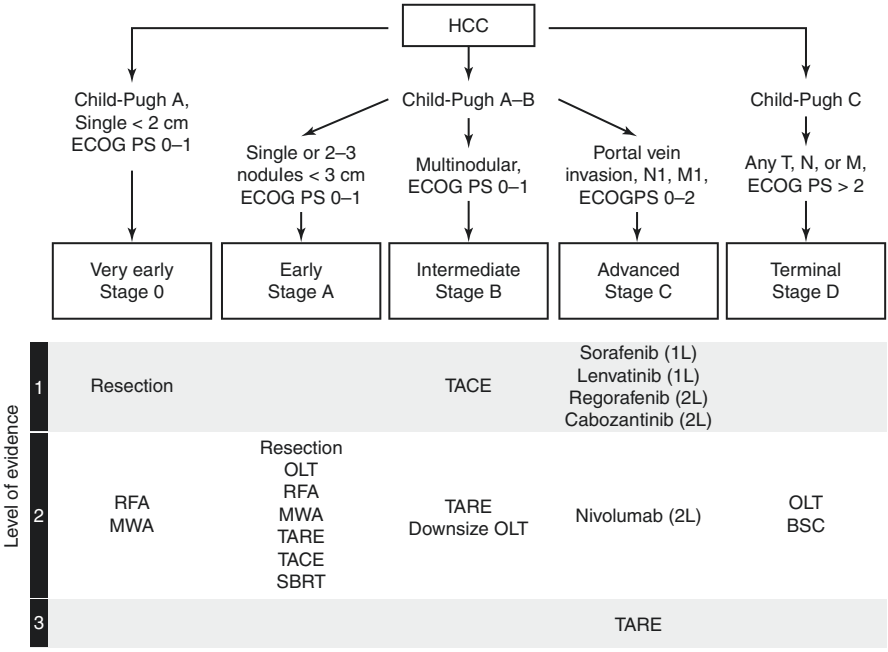

Fig. 8.1 American Association for the Study of Liver Diseases (AASLD) HCC staging and treatment recommendation. HCC, hepatocellular carcinoma; ECOG, Eastern Cooperative Oncology Group; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; MWA, microwave ablation; OLT, orthotopic liver transplantation; TARE, transarterial radioembolization; SBRT, stereotactic body radiotherapy; BSC, best supportive care. (Redrawn based on Marrero et al. [5])

The criteria in place to help guide liver transplant allocation are even more specific, and the organs available are scarce. In an attempt to facilitate fair allocation of donor livers, the United Network for Organ Sharing (UNOS) was established and ranks patients for organ allocation based on a pretransplant mortality risk, or Model for End-Stage Liver Disease (MELD) score [11]. Unfortunately, relatively few eligible HCC patients receive a liver transplantation due to the limited supply of donor organs. When orthotopic liver transplantation (OLT) was first introduced, it was offered to patients with unresectable disease secondary to tumor burden or liver dysfunction, and in so doing, the survival rates were low and recurrence rates were high [12, 13]. In an attempt to improve survival following OLT, the Milan criteria were established [14]. The Milan criteria apply to patients with a single nodule ≤5 cm or up to three nodules ≤3 cm and without evidence of macroscopic vascular invasion, lymph node involvement, or extrahepatic disease [14]. The University of California San Francisco (UCSF) criteria were subsequently created in an attempt to broaden the Milan criteria, which were felt to be too restrictive [15]. The UCSF criteria include a single nodule ≤6.5 cm or ≤3 nodules with the largest being ≤4.5 cm, with a total tumor burden ≤8 cm [15]. The Milan criteria remain the most frequently applied criteria for liver allocation, with 97% of all transplanted livers from 2002 to 2007 meeting Milan criteria, while only 3% fit UCSF criteria [16].

Surgical Resection

AASLD stage 0/A patients clearly meet criteria for resection of their HCC. AASLD stage 0 constitutes patients with a single nodule ≤2 cm with preserved liver function, while AASLD stage A includes a solitary lesion or up to three nodules ≤3 cm with preserved liver function and performance status. The goal of hepatic resection for HCC is to appropriate oncological margins while maintaining a functional liver remnant.

Preoperative Assessment

HCC is a predominantly radiographic diagnosis, and most guidelines do not recommend routine biopsy of suspected HCC lesions in cirrhotic patients. Appropriate staging is necessary prior to proceeding with resection. Extrahepatic staging would include CT of the chest and CT or MRI of the abdomen and pelvis [17]. Additionally, assessment of the volume of the future liver remnant (FLR) is imperative, due to the risk of post-hepatectomy liver failure (PHLF) following resection in cirrhotic patients. PHLF is a major cause of morbidity and mortality following hepatectomy, and therefore accurate estimation of the remnant functional liver volume is one of the most important preoperative steps in reducing postoperative morbidity [18].

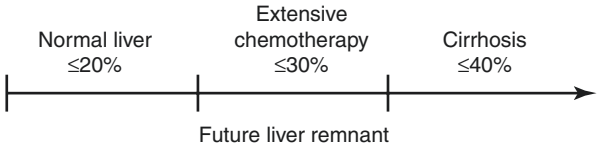

Fig. 8.2 Guidelines for minimum standardized future liver remnants following hepatectomy stratified by underlying liver condition. (Reprint from Zorzi et al. [25])

Knowledge of standard liver volume proportions is necessary to determine if extent of surgical resection will result in an appreciable decline in remnant functional liver volume. On average, the right liver (segments V–VIII) contributes two-thirds of the total liver volume, while the left liver (segments II–IV) contributes one-third of the total liver volume [19]. The method used to calculate the future liver remnant (FLR) is heavily debated and can be calculated based on formulas involving body surface area and radiographic imaging [20, 21]. Computed tomography with 3D reconstruction or volumetric MRI traces the hepatic segmental contours and multiplies the surface area by slice thickness to calculate the total liver volume [22]. To calculate the FLR, the following formula is used: (resected volume − tumor volume)/(total liver volume − tumor volume) [23, 24]. Guidelines for minimum standardized future liver remnants before hepatic resection have been well defined in studies for hepatic resection of colorectal metastases (Fig. 8.2).

The minimum FLR considered to be safe following extended liver resection is based on the function and exposure status of the underlying liver. Normal livers without cirrhosis are considered low risk for PHLF if 20% of the liver volume remains following resection, while livers exposed to extensive chemotherapy and cirrhotic livers necessitate greater liver remnants of 30% and 40%, respectively [25] Small liver remnant volume is associated with higher rates of PHLF and other complications following hepatectomy [26, 27].

If the preoperative assessment of FLR is considered to be inadequate or borderline, several options are available to induce hypertrophy of the remnant liver and thus increase the FLR following resection. Portal vein embolization (PVE) is the most commonly utilized adjunct and most often involves ipsilateral portal vein puncture (although contralateral or ileocolic approaches are also feasible) with subsequent embolization of the main portal vein to the involved lobe using the institutional preferred embolic material [28]. Embolization results in progressive ischemia and necrosis with a subsequent increase in flow to the unaffected lobe and resultant growth factor and cytokine release stimulating parenchymal hypertrophy. A prospective trial compared upfront right hepatectomy to PVE followed by right hepatectomy and found significantly reduced postoperative complications in the PVE cohort [29].

Typically, repeat imaging is performed 4–6 weeks following PVE, and the degree of hypertrophy and the growth rate are strong predictors of PHLF following hepatectomy [28]. In a study from Memorial Sloan Kettering, no patient with a growth rate

of >2.66% volume per week developed PHLF, concluding that early surgery may be safe in patients who demonstrate an adequate growth rate [28]. It is accepted that the absence of early hypertrophy following a successful PVE is an indication of low regenerative capacity and a contraindication to major hepatectomy [30]. In cases of unsuccessful hypertrophy from PVE alone, some studies recommend a dual inflow obstruction technique with the addition of hepatic artery embolization [30, 31].

Associated liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a recently developed strategy to prevent PHLF by inducing rapid remnant liver hypertrophy [32, 33]. The first step of the ALPPS procedure is to ligate a branch of portal vein to induce hepatic hypertrophy. After confirming increased hepatic volume typically 1–2 weeks after the initial surgery, HCC tumor resection is performed. Limited hypertrophy was noted when substantial fibrosis is present in the liver [34]. In a series of 17 HCC patients at an Italian center, 2-year postsurgical overall survival and disease-free survival were 38.5% and 60%, respectively [35].

Choice of Surgical Resection

For small tumors measuring <2 cm, most surgeons advocate for anatomic resections over nonanatomic (wedge) resections due to the limitations of preoperative imaging in diagnosing concomitant satellite lesions or portal vein involvement that would preclude nonanatomic resections. A recent meta-analysis comparing anatomic to nonanatomic resections found no difference in postoperative morbidity, including PHLF, and found a lower recurrence rate and higher 5-year disease-specific survival (DSS) in the anatomically resected cohort (OR 0.27, 95% CI 0.2–0.4 for recurrence in the anatomic group compared to nonanatomic cohorts, and OR 2.1, 95% CI 1.4–3.1 for DSS for the anatomic compared to nonanatomic cohorts) [36].

For larger tumors measuring 2–5 cm, the most parenchyma-preserving anatomic resection is recommended in an effort to avoid postoperative outcomes. Table 8.1 summarizes the outcomes of studies evaluating more extensive anatomic resections to parenchyma-preserving anatomic resections. While the results of these studies vary, there appears to be no difference in survival between parenchyma-sparing resections and extended hepatectomy with significant advantages in postoperative outcomes [37–40]. We prefer to preserve as much of the future liver as possible and therefore recommend parenchyma-sparing resections over extended hepatectomy when R0 resections can be achieved. For much larger tumors measuring >5 cm or with poor prognostic features, and hence outside of AASLD stage 0/A criteria, there is evidence that surgical resection is efficacious. In such cases, major liver resections such as right hepatectomy and extended right and left hepatectomy are often necessary. Figure 8.3 depicts the nomenclature of and liver segments in various anatomic liver resections [19].

Table 8.1 Summary of studies comparing parenchyma-preserving hepatectomy to formal or extended hepatectomy

Author, year	No. of patients	Findings	5-year OS	DSS
Right-sided tumors				
Fisher, 2013 [37]	RPS = 100 RH = 480	↑ PHLF in RH (1% RPS vs 9% RH, $p < 0.001$)	n/a	n/a
Yip, 2015 [38]	RPS = 49 RH = 32	No difference PHLF (2% RPS vs 9% RH, $p = 0.34$)	(84% RPS vs 76% RH, $p = 0.77$)	(52% RPS vs 53% RH, $p = 0.86$)
Central tumors				
Lee, 2015	*Matched* CH = 63 EH = 63	↑ Pringle time in CH (50 vs 36 min, $p = 0.04$) ↓ Blood loss in CH (800 vs 500 mL, $p = 0.01$) A post-op bilirubin > 4 mg/dL was observed in 2% CH vs 39% EH ($p < 0.01$)	n/a	n/a
Chen, 2014 [40]	CH = 118 RE = 47 LE = 33	↑ PHLF in RE (11% CH vs 2% RE, $p = 0.03$) but no difference between CH and LE ↓ Rate of R0 resection in CH (67% vs 83% RE and 85% RE, $p < 0.05$)	(29% CH vs 30% EH, $p = 0.58$)	(17% CH vs 27% EH, $p = 0.11$)
Lee, 2014 [100] systemic review of CH cases	CH = 895	Operative time = 115–627 min Pringle was used in majority of cases Intraop blood loss = 380–2450 mL	32–67%	17–32%

RPS right posterior sectionectomy, *RH* right hepatectomy, *CH* central hepatectomy, *EH* extended hepatectomy, *RE* right extended hepatectomy, *LE* left extended hepatectomy, *OS* overall survival, *DSS* disease-specific survival

Surgical Technique

For large right-sided tumors, two approaches have been proposed. The conventional approach involves inflow vascular control followed by complete mobilization of the right lobe of the liver with control of the right hepatic vein prior to parenchymal transection [41–43]. Advocates of this technique report reductions in intraoperative blood loss [41–43]. However this technique is not always feasible when resecting very large tumors such as those that surround vascular structures or if the sheer size of the tumor may greatly limit access to the hepatic vein.

The anterior approach to major right hepatectomy is an alternative technique that involves initial parenchymal transection before the right lobe of the liver is mobilized. A randomized clinical trial compared anterior and conventional approach for right hepatectomy for HCC > 5 cm and found less intraoperative blood loss and decreased transfusion rate and improved OS in the anterior approach (median OS >

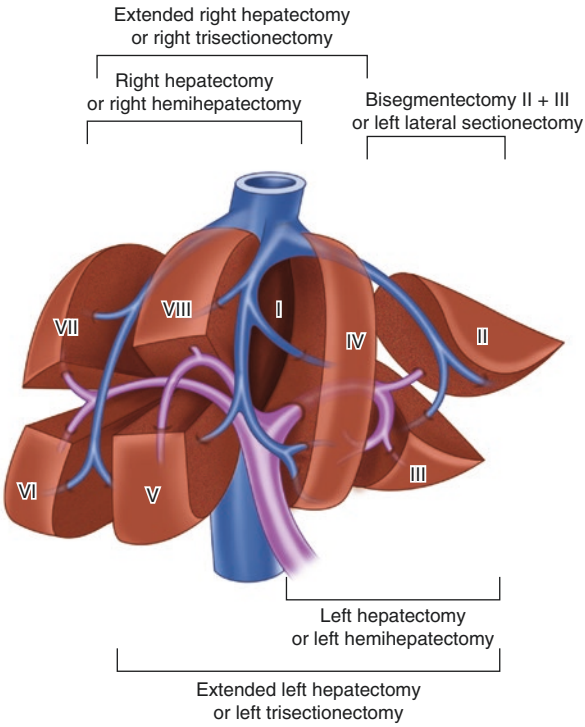

Fig. 8.3 Nomenclature of and liver segments involved in anatomic liver resections. (Reprint from Abdalla et al. [19])

68 months compared to 23 months, $p = 0.01$) [44]. Although this trial is associated with significant methodological issues, other studies have replicated these findings and further support utilizing an anterior approach to major right hepatectomy [45, 46]. An adjunct to the anterior approach includes the use of Belghiti's hanging maneuver. This maneuver is performed by creating a retrohepatic tunnel anterior to the inferior vena cava and securing a Penrose drain or umbilical tape to hang the liver and guide the transection plane [47] (Fig. 8.4). We recommend conventional approach for the majority of major right hepatectomy but do endorse the use of the anterior approach with or without the use of hanging technique when mobilization of the right lobe of the liver is not feasible, particularly for tumors >5 cm or with invasive characteristics.

Surgical Outcomes

An R0 resection margin is critical for a complete oncologic resection. A meta-analysis evaluating the influence of the width of resection margin on recurrence and survival for HCC showed that a resection margin ≥1 cm did not provide significant prognostic benefit compared with a resection margin <1 cm [48]. We recommend microscopically negative resection margin and do not specify a margin width.

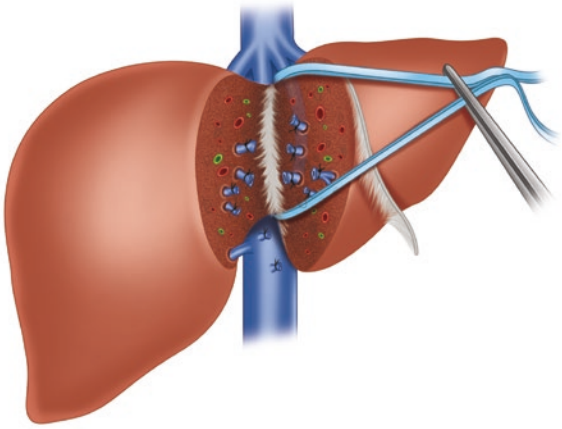

Fig. 8.4 Hanging maneuver as an adjunct to the anterior approach for major right hepatectomy. Umbilical tape is passed retrohepatically, anterior to the inferior vena cava, and used to lift the liver and facilitate parenchymal transection. (Reprint from Belghiti et al. [47])

The largest study to date reports 1-, 3-, and 5-year survival rates of 85%, 64%, and 45%, respectively [49]. Five-year survival rates as high as 60–70% have been reported in Child-Pugh stage A patients with tumors <2 cm undergoing surgical resection [5]. The main predictors for disease recurrence and survival following liver resection include tumor number, tumor size, vascular invasion, and tumor grade. In multifocal HCC, more than three nodules, positive margin, and microvascular invasion are associated with worse overall survival [50].

Recurrence is reported in up to 70% of patients at 5 years post resection [51–54]. Recurrence occurring within 2 years of resection is thought to be secondary to disease propagation from the original resected liver tumor [55]. Factors such as tumor size, microvascular invasion, satellite nodules, and nonanatomic resection are likely causes of early recurrence [56]. Recurrence more than 2 years post resection has been defined as a delayed recurrence and may represent "de novo" tumors in an oncogenic cirrhotic liver [57, 58]. Risk factors associated with delayed recurrence include underlying cirrhosis, active hepatitis, vascular invasion, moderate or poorly differentiated HCC, and multi-nodularity [55]. The role of genomic profiling of molecular signatures to define the level of oncogenicity in the cirrhotic liver has been proposed, but further evidence is needed prior to clinical implementation [59].

Novel Approaches to Surgical Resection

Over the last two decades, minimally invasive surgical approaches have become increasingly utilized. There is abundant literature within colorectal surgery, gynecology, and urology to demonstrate similar oncologic outcomes with possible improved postoperative morbidity compared to an open approach. The limitations of minimally invasive techniques become more apparent with increasing case complexity; thus implementation into hepato-pancreatico-biliary operations has

been slower than in the aforementioned specialties. With conventional laparoscopy, there is reduced visualization and the range of motion is restricted to 4° of freedom compared to 7° of freedom of the human wrist [60, 61]. However, the introduction of robotic surgical platforms with its enhanced range of motion and better visualization has allowed a more natural transition into minimally invasive liver operations. Currently, laparoscopic, hand-assisted laparoscopic, hybrid approach with laparoscopic mobilization and open parenchymal transection, and robotic approaches are utilized in liver resections.

Laparoscopic liver resection has been used with increased frequency and is mostly used for nonanatomic liver resections. A large study of 2800 laparoscopic liver resections demonstrated that nearly two-thirds of the cases were laparoscopic wedge resection or left lateral sectionectomy. The conversion rate was 4% in this series. The overall complication rate was 11% with the most common complications being bile leak (1.5%) and PHLF (1%). Comparative studies of laparoscopic to open approaches have demonstrated increased operative time with a reduction of perioperative blood loss and postoperative morbidity and shorter length of stay in the laparoscopic groups [45, 62–66]. Oncologic outcomes are similar between laparoscopic and open approaches [45, 66].

The largest series utilizing the robotic surgical platform is from Tsung et al. which compared 57 robotic- to 114 laparoscopic-assisted hepatic resections. There was no significant difference in intraoperative or postoperative outcomes with the exception that the robotic approach was associated with a higher rate of completion by a minimally invasive approach (81% vs 7.1%, $p < 0.05$) [67]. However, a meta-analysis pooled seven studies and found laparoscopic liver resection resulted in reduced blood loss and shorter surgical times compared to robotic liver resections without differences in conversion rate, margin status, or morbidity [68].

Although there is no clear evidence to guide choice of minimally invasive modality, we feel that the literature supports the use of minimally invasive approaches at high-volume centers. The proposed learning curve for laparoscopic liver resections is 60 cases and no case volume proposal is available for robotic approach [69]. Relative contraindications for minimally invasive major liver resection include prior major abdominal surgeries; chronic obstructive pulmonary disease; lesions near the vena cava, hilum, or major hepatic veins; and patients with coagulopathy or portal hypertension [70]. Absolute contraindications include patients unable to tolerate pneumoperitoneum and patients with severe portal hypertension [70].

Role of Adjuvant Therapy

Adjuvant therapy following curative resection is an unmet need to improve postsurgical prognosis. Due to the high rate of recurrence following resection, attempts have been made to find adjuncts to resection in an effort to improve recurrence-free survival. Recurrence can occur due to dissemination of resected primary tumor

(disseminative recurrence) or de novo carcinogenesis in remnant diseased livers (de novo recurrence), and precise prediction of these distinct types of recurrence will enable tailored preventive intervention accordingly [58]. Sorafenib, an oral multikinase inhibitor, is approved for use in patients with unresectable HCC based on two randomized controlled trials [71]. Due to its proven efficacy in advanced HCC, the STORM trial was designed to assess the efficacy and safety of sorafenib versus placebo as adjuvant therapy after successful surgical resection or local ablation. No difference was noted in median recurrence-free survival between the groups, and it was concluded that adjuvant sorafenib is not an effective treatment for HCC following resection or ablation [72].

For patients with underlying viral hepatitis, interferon (IFN) has been proposed as a treatment adjunct. IFN suppresses the replication of HBV and HCV and elicits a tumoricidal effect on HCC tumor cells [73–75]. Multiple RCTs have been completed with conflicting conclusions. A meta-analysis of seven randomized controlled trials with 620 patients demonstrated adjuvant IFN treatment was associated with a significant reduction in 2-year mortality risk reduction of 0.65 (95% CI 0.5–0.8) [76]. IFN was also associated with a significant risk reduction in tumor recurrence of 0.86 (95% CI 0.8–0.9) [76]. Two subsequent meta-analyses had similar conclusions [77, 78]. Due to methodological limitations of the randomized trials, no definitive recommendations have been made to date.

Liver Transplantation

The first liver transplant was performed by Thomas Starzl in 1963; however it was not until after 1980, with the introduction of cyclosporine, in addition to azathioprine, prednisone, and antilymphocyte globulin, that patients were able to consistently achieve 1-year survival [79]. This improvement in survival resulted in expansion of liver transplantation nationally. Since 2001, HCC has significantly increased as a major cause of liver transplantation, and in 2012 it surpassed hepatitis C virus as the most common indication for liver transplantation [80]. Liver transplantation is a popular therapeutic option for HCC because it not only removes the macroscopic tumors and microscopic foci of HCC but it also replaces the underlying neoplastic liver and prevents the development of complications associated with cirrhosis [56].

Preoperative Assessment

The role for transplantation is limited to patients with low disease burden as literature has shown patients with small tumors can be cured, while patients with extensive disease have poor outcomes [14]. The Milan criteria are the benchmark for the selection of HCC patients for liver transplantation [17]. Accurate extrahepatic

staging is necessary and would include CT of the chest and CT or MRI of the abdomen and pelvis [17].

In 2002, UNOS changed the allocation prioritization of eligible recipients from a Child-Pugh categorized system to a MELD-based system which provides a more accurate predictor of waitlist mortality compared to Child-Pugh and is the current practice today [81]. Even still, patients placed on the transplant list with HCC meeting Milan criteria often have sufficient liver function, and therefore their MELD score often underrepresents their survival prognosis [82]. For this reason, exception MELD (eMELD) points are given to HCC patients listed within the Milan criteria [82]. The eMELD points given to eligible patients are 22, equivalent to a 15% probability of death within 3 months, and are increased every 3 months until transplantation or until the patient no longer meets Milan criteria [82]. Patients with HCC must undergo CT or MRI scanning, and alpha-fetoprotein measurements every 3 months to document these still are within Milan criteria [17, 82]. Patients found to have progressed beyond Milan criteria should be placed on hold and considered for downstaging, and patients with progressive disease in whom locoregional intervention is not appropriate should be removed from the waiting list [17].

Extending Beyond the Milan Criteria: The UCSF Criteria and the Metroticket Calculator

There has been a move to extend transplant allocation to patients exceeding Milan criteria. Proponents of extension claim that the improved accuracy of imaging techniques has enabled identification of very small lesions that were not detectable 10 years ago and thus resulted in exclusion of patients who would have historically been listed [83]. The UCSF criteria are the most widely used criteria outside of the Milan criteria, but most studies report worse overall survival when comparing the two criteria [84, 85]. Some studies conclude that measuring response to neoadjuvant therapy or downstaging should be used as an adjunct to support listing patients within UCSF criteria [86]. Others suggest UCSF criteria are more suitable for cases of liver transplantation in living donors but should not be used for listing of deceased donor livers [85].

In an effort to more accurately define criteria exceeding Milan that could be utilized in liver allocation, Mazzaferro et al. developed an algorithm known as the Metroticket calculator to identify transplant patients exceeding Milan criteria who would have similar survival to those who are within Milan criteria, i.e., a 70% 5-year overall survival rate [83]. The authors concluded that patients without microvascular invasion who fell within the up-to-seven criteria (seven as the sum of the size of the largest tumor in centimeters and the number of tumors) achieved similar OS to patients meeting Milan criteria [83]. They argue that the up-to-seven criteria should be considered to extend liver transplantation to more HCC patients [83]. However, the Milan criteria remain the recommended criteria to determine transplant-eligible HCC patients.

Surgical Technique of Conventional Liver Transplant

The conventional technique utilized in liver transplantation is the bicaval anastomosis (Fig. 8.5a) [79]. This is created by performing the recipient hepatectomy en bloc with the native inferior vena cava and replacing with the donor liver and IVC. To perform the donor hepatectomy, the components of the hepatic hilum are isolated. The left and right hepatic arteries are divided separately. The proper hepatic artery is dissected proximal to the origin of the gastroduodenal artery as a patch composed of the GDA, and common hepatic artery is used for the subsequent arterial anastomosis. The cystic duct and the common bile duct are divided. The portal vein is dissected to the level of the first pancreatic branch. Prior to division of the portal vein, the liver is mobilized completely. Once mobilization is complete, the portal vein, the infrahepatic IVC, and the suprahepatic IVC are clamped, and the liver is removed. Some surgeons prefer to utilize venous bypass during the anhepatic phase. Two end-to-end IVC anastomoses are required, one suprahepatic and one infrahepatic [56, 80].

Most commonly, end-to-end portal vein, hepatic artery, and common bile duct anastomoses are also performed. Technical considerations of the remaining anastomoses include maintaining "growth factor" when tying a circumferentially run suture in the PV anastomosis which acts to accommodate space for blood flow and decrease the likelihood of stricture and subsequent thrombosis. The arterial anastomosis includes a Carrel patch on the donor and recipient ends, typically creating a patch composed of the donor celiac trunk and aorta to a patch composed of the recipient GDA and the proper hepatic artery. The biliary reconstruction can be performed via an end-to-end choledochocholedochostomy or a Roux-en-Y hepaticojejunostomy, although the Roux-en-Y approach is often reserved for re-transplantation, strictured bile ducts, or living donor transplants. Choledochocholedochostomy is preferred if possible as it allows preserved function of the sphincter of Oddi and allows easy endoscopic access to the biliary system for diagnostic and therapeutic purposes [56, 80]. The donor liver can also be divided to create split liver transplantation, in which the deceased right donor liver is transplanted into an adult recipient and the deceased left donor liver is transplanted into a pediatric recipient (Fig. 8.5c).

Piggyback Technique of Liver Transplantation

When the recipient IVC is preserved, the anastomosis is performed in a "piggyback" technique (Fig. 8.5b). The recipient hepatectomy involves complete mobilization of the liver off the IVC, including ligation of all hepatic veins. The donor suprahepatic IVC is anastomosed to the confluence of the recipient hepatic veins. The remaining anastomoses are performed similar to the conventional technique. Modifications of

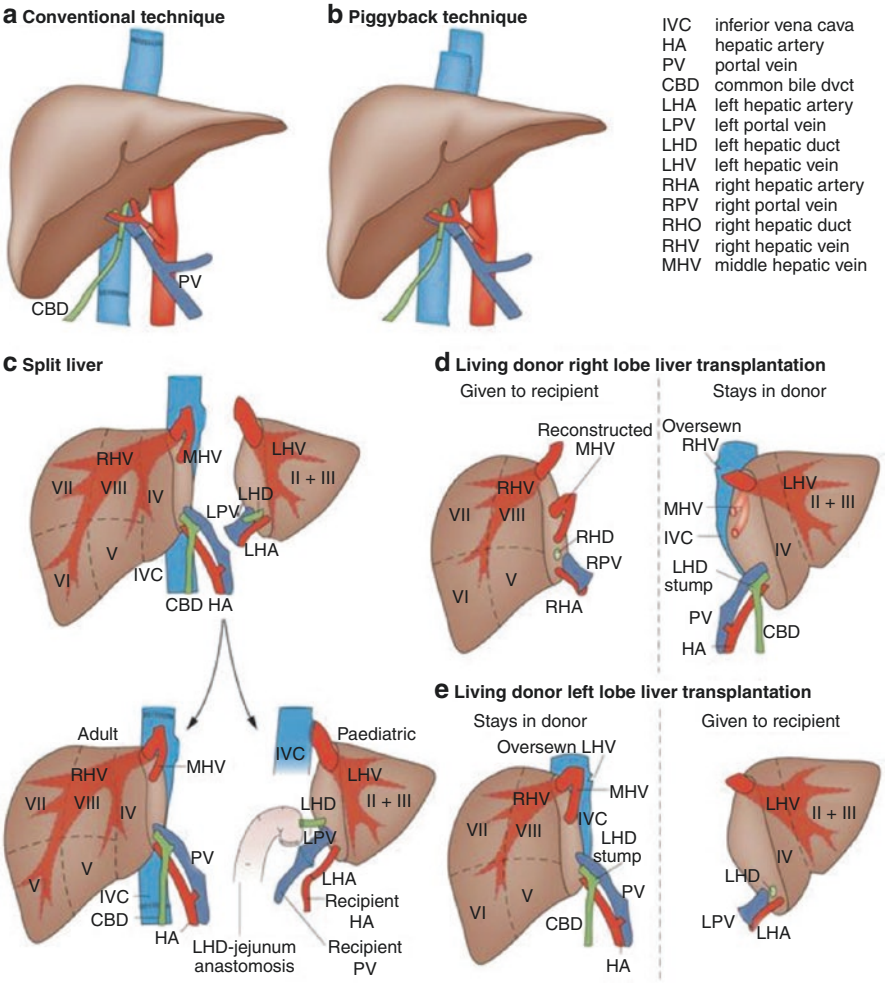

Fig. 8.5 Piggyback technique of liver transplantation. (**a**) Depiction of a conventional bicaval anastomosis transplantation. The donor IVC is placed as an interposition segment of the transected recipient infrahepatic and suprahepatic IVC, and two IVC anastomoses are created in an end-to-end fashion. (**b**) Depiction of a piggyback transplantation. The recipient IVC remains intact and the donor suprahepatic IVC is sutured to the confluence of the hepatic veins on the suprahepatic recipient IVC. (**c**) Depiction of split liver transplantation, where a single deceased donor liver is divided to provide two donor organs. (**d**) Depiction of living donor right lobe liver transplantation, with isolation of the right +/− middle hepatic vein, right hepatic duct, right hepatic artery, and right portal vein. (**e**) Depiction of living donor left lobe liver transplantation, with isolation of the left hepatic vein, left hepatic duct, left hepatic artery, and left portal vein. (Reprint from Zarrinpar and Busuttil [79])

the piggyback technique include a side-to-side between the donor IVC and the recipient IVC or an end-to-side anastomosis between the suprahepatic donor IVC and the recipient suprahepatic IVC by extending the common orifice of the hepatic veins [87].

Surgical Technique of Living Donor Liver Transplant

Living donor liver transplant (LDLT) was first performed in the pediatric population and, with its success, was introduced into the adult population. It is an attractive option because it can help offload national organ shortages, it provides an opportunity for transplantation prior to liver decompensation, the cold ischemic time is significantly reduced, and there is a possible immunologic advantage to receiving an organ from a haploidentical sibling or patient [88]. The donor liver can be provided by a right or left hepatectomy. For the donor right hepatectomy, the first step is to take down the falciform ligament toward the hepatic veins and develop the sulcus between the right and middle hepatic veins (Fig. 8.5d). A cholecystectomy is performed and an intraoperative cholangiogram is captured to determine the anatomy of the right biliary system. The hilum is subsequently dissected, isolating the right hepatic artery, right common hepatic duct, and right portal vein. The right lobe of the liver is mobilized by separating its ligamentous attachments and exposing the retrohepatic IVC and dividing the short hepatic veins of the right lobe of the liver. The right hepatic vein is isolated and encircled with a vessel loop [88].

The parenchymal transection line is defined by occluding the vascular inflow and marked with electrocautery, ensuring the transection line is 2 cm from the middle hepatic vein. The parenchyma is divided according to surgeon preference. The right hepatic duct is sharply divided close to the liver to avoid injury to the left biliary system. The right hepatic artery, portal vein, and hepatic vein are divided. With this division, the donor hepatectomy is free, allowing removal and preparation of the donor organ by flushing the vascular and biliary structures [88]. A donor left hepatectomy is performed similarly, but isolating the left liver lobe structures (Fig. 8.5e).

Recipient hepatectomy is performed using the piggyback technique, preserving the retrohepatic IVC. The implantation can be performed by anastomosing the donor right hepatic vein to the recipient right hepatic vein or to the confluence of the hepatic veins [88]. The remaining anastomoses are performed as above with the exception of the biliary reconstruction which necessitates a Roux-en-Y hepaticojejunostomy [88].

Surgical Outcomes

HCC patients who undergo transplantation within Milan criteria have a recurrence rate of 10%. The 1-year and 5-year survival rates after liver transplantation approach 85% and 70% for deceased donor liver transplantation. There is evidence that the

piggyback technique is associated with shorter operative time, shorter anhepatic phase, shorter warm ischemia time, and fewer transfusions compared to the conventional approach. Additionally, the piggyback technique has been associated with improved overall survival and recurrence-free survival [89]. In a propensity-matched analysis, LDLT had significantly higher rates of perioperative complications, most often biliary complications compared to deceased donor liver transplantation (DDLT), while DDLT was more likely to have serious complications leading to graft loss. The survival rates were similar for the two techniques, and LDLT appears to be a valuable alternative to DDLT at high-volume transplant centers [90].

Management of Posttransplant Complications

Biliary complications are the most common posttransplant complication and include bile duct strictures and biliary leak. Strictures occur in 15% of cases, and treatment depends on location of the biliary stricture [91]. Strictures occurring at the biliary anastomosis can be early or late onset. Early-onset anastomotic strictures are often due to technical issues, while late-onset anastomotic strictures are related to ischemic changes resulting in fibrosis [80]. Nonanastomotic strictures result from ischemia. ERCP is the best diagnostic modality as it also allows therapeutic balloon dilation and stent placement for anastomotic strictures. Management of nonanastomotic strictures includes stenting and, if the stricture is limited to the extrahepatic ducts, consideration of revising to a hepaticojejunostomy [91]. Bile leak occurs in 20% of liver transplantations. Most are self-limiting with adequate drainage. If patients demonstrate signs of systemic decompensation, an ERCP with sphincterotomy and stent placement should be considered. It is rare that a bile leak should need operative revision [91].

Hepatic artery thrombosis occurs at a rate of 2–5%, and half occur <30 days after surgery (early onset), while the other half occur after 30 days (late onset). Early-onset thrombosis leads to graft loss. If caught early, it can be treated with thrombectomy and reconstruction of the arterial anastomosis or re-transplanted. In late-onset thrombosis, the graft will often survive due to portal venous flow; however the biliary system is completely dependent on arterial blood flow and is more sensitive to arterial injury. Duplex ultrasound is used to evaluate the patency of the hepatic artery, and a flow less than 200 cc/min is associated with an increased risk of hepatic artery thrombosis and primary non-function [92, 93]. Hepatic artery stenosis occurs in 2–10%, with early onset occurring in 40% and late onset in 60% of patients. Patients with hepatic artery stenosis often have an insidious course, making it difficult for diagnosis. Therapeutic interventions include surgical revision or percutaneous angioplasty with or without stent placement.

Portal vein thrombosis and stenosis occur around 1% of the time and are more common in living donor transplants. Risks of portal vein thrombosis include the recipient portal vein thrombus necessitating intraoperative thrombectomy, hypercoagulable state, low-flow states, and small vein diameter. Portal vein thrombosis

results in early graft loss. Duplex ultrasound is used to diagnose portal vein thrombosis and should measure greater than 1 L/min [93]. Like arterial strictures, venous strictures can be managed with revision or percutaneous angioplasty.

Postoperative Management of Recurrent HCC Following Transplantation

There is no strong evidence to suggest surveillance imaging or measurement of alpha-fetoprotein is necessary following liver transplantation for HCC [17]. There is no indication for re-transplantation of recurrent HCC following transplantation, but recurrence may be treated by alternative locoregional or systemic modalities [17].

Comparison of Resection and Transplantation

The optimal treatment for early-stage HCC remains controversial. Studies comparing the two treatment modalities have resulted in mixed conclusions. A propensity score-matched analysis from Shen demonstrated comparable long-term survival in all patients meeting Milan criteria treated with resection or transplantation, with the exception of a subgroup of patients with AFP ≥ 400 ng/mL, in whom an associated survival advantage was seen with transplantation [94]. Additional larger propensity-matched analyses demonstrated an associated improvement in long-term survival with transplantation compared to resection [95]. A meta-analysis of all available literature demonstrated low quality of evidence demonstrating that resection is a good alternative to liver transplantation in eligible patients with early-stage HCC. To date, there are no randomized controlled trials [96]. Figure 8.6

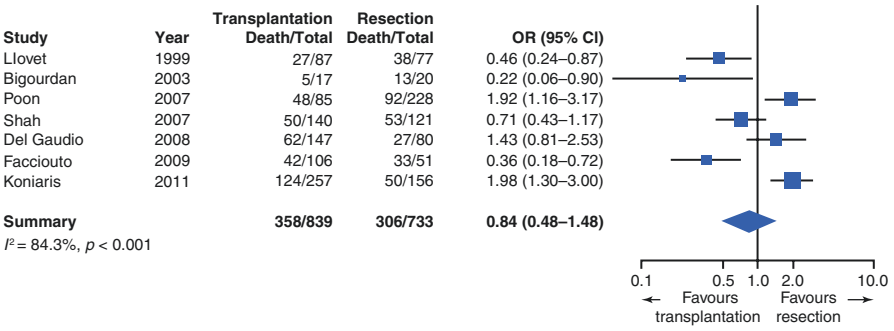

Fig. 8.6 Meta-analysis of seven studies comparing transplantation and resection. The data represents 5-year overall survival data and odds ratio signifying survival of resection to transplantation with 95% confidence intervals. The combined odds ratio is not statistically significant of 0.84 (95% CI 0.48–1.48). (Reprinted from Proneth et al. [96])

represents the meta-analysis comparing survival following surgical resection to survival following liver transplantation. There was no significant difference in survival in the pooled analysis [96].

HCC recurs or develops de novo in 50–80% of patients at 5-years post liver resection [51]. Recurrence rates following liver transplantation are much lower, reported at 4–10% if transplantation is performed within Milan criteria [14]. Recurrence in a native liver following surgical resection decreases the 5-year survival rates from 50–80% to 39–48% [51, 97]. Recurrence in a transplanted liver decreases the 5-year overall survival rate from 95% to 60% [51]. The most thorough comparison of resection and transplantation includes an intention-to-treat analysis and thus incorporates drop-out time associated with the transplant waiting list. Studies have demonstrated that the survival benefit of transplantation for HCC is most significant when patients wait less than 6 months from the time of listing [5]. Koniaris performed an intention-to-treat comparison of resection and transplantation, finding 1-year and 5-year survival of 92.0% and 63.0% for resection and 83.0% and 41.0% for transplant ($p = 0.036$) [98]. For this reason, some suggest that transplantation is the preferred treatment of choice for early-stage HCC, but resection should be the preferred option in regions where access to transplant is not available or is expected to exceed 6 months [99]. There is no strong evidence favoring one surgical treatment over the other. We consider both to be excellent options with comparable outcomes.

References

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study. JAMA Oncol. 2018;4:1553–68. https://doi.org/10.1001/jamaoncol.2018.2706.
2. Ioannou GN, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2007;5(8):938–45, 45 e1–4. https://doi.org/10.1016/j.cgh.2007.02.039.
3. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009;27(9):1485–91. https://doi.org/10.1200/JCO.2008.20.7753.
4. Yopp AC, Mansour JC, Beg MS, Arenas J, Trimmer C, Reddick M, et al. Establishment of a multidisciplinary hepatocellular carcinoma clinic is associated with improved clinical outcome. Ann Surg Oncol. 2014;21(4):1287–95. https://doi.org/10.1245/s10434-013-3413-8.
5. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology. 2018;68(2):723–50. https://doi.org/10.1002/hep.29913.
6. Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. Ann Surg. 1999;229(6):790–9; discussion 799–800.
7. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. Hepatology. 1999;30(6):1434–40. https://doi.org/10.1002/hep.510300629.

8. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28(3):751–5. https://doi.org/10.1002/hep.510280322.
9. Okuda K. Natural history of hepatocellular carcinoma including fibrolamellar and hepatocholangiocarcinoma variants. J Gastroenterol Hepatol. 2002;17(4):401–5.
10. Kamarajah SK, Frankel TL, Sonnenday C, Cho CS, Nathan H. Critical evaluation of the American Joint Commission on Cancer (AJCC) 8th edition staging system for patients with hepatocellular carcinoma (HCC): a surveillance, epidemiology, end results (SEER) analysis. J Surg Oncol. 2018;117(4):644–50. https://doi.org/10.1002/jso.24908.
11. Martin AP, Bartels M, Hauss J, Fangmann J. Overview of the MELD score and the UNOS adult liver allocation system. Transplant Proc. 2007;39(10):3169–74. https://doi.org/10.1016/j.transproceed.2007.04.025.
12. Bismuth H, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. Ann Surg. 1993;218(2):145–51.
13. Sarpel U, Schwartz M. Liver transplantation for hepatocellular carcinoma. Hepatol Res. 2007;37(Suppl 2):S264–6. https://doi.org/10.1111/j.1872-034X.2007.00194.x.
14. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334(11):693–9. https://doi.org/10.1056/NEJM199603143341104.
15. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology. 2001;33(6):1394–403. https://doi.org/10.1053/jhep.2001.24563.
16. Patel SS, Arrington AK, McKenzie S, Mailey B, Ding M, Lee W, et al. Milan criteria and UCSF criteria: a preliminary comparative study of liver transplantation outcomes in the United States. Int J Hepatol. 2012;2012:253517. https://doi.org/10.1155/2012/253517.
17. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. Lancet Oncol. 2012;13(1):e11–22. https://doi.org/10.1016/S1470-2045(11)70175-9.
18. Itoh S, Yoshizumi T, Shirabe K, Kimura K, Okabe H, Harimoto N, et al. Functional remnant liver assessment predicts liver-related morbidity after hepatic resection in patients with hepatocellular carcinoma. Hepatol Res. 2017;47(5):398–404. https://doi.org/10.1111/hepr.12761.
19. Abdalla EK, Denys A, Chevalier P, Nemr RA, Vauthey JN. Total and segmental liver volume variations: implications for liver surgery. Surgery. 2004;135(4):404–10. https://doi.org/10.1016/j.surg.2003.08.024.
20. Ribero D, Chun YS, Vauthey JN. Standardized liver volumetry for portal vein embolization. Semin Intervent Radiol. 2008;25(2):104–9. https://doi.org/10.1055/s-2008-1076681.
21. Urata K, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, et al. Calculation of child and adult standard liver volume for liver transplantation. Hepatology. 1995;21(5):1317–21.
22. Madoff DC, Abdalla EK, Vauthey JN. Portal vein embolization in preparation for major hepatic resection: evolution of a new standard of care. J Vasc Interv Radiol. 2005;16(6):779–90. https://doi.org/10.1097/01.RVI.0000159543.28222.73.
23. Kubota K, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, et al. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. Hepatology. 1997;26(5):1176–81. https://doi.org/10.1053/jhep.1997.v26.pm0009362359.
24. Ogasawara K, Une Y, Nakajima Y, Uchino J. The significance of measuring liver volume using computed tomographic images before and after hepatectomy. Surg Today. 1995;25(1):43–8.
25. Zorzi D, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. Br J Surg. 2007;94(3):274–86. https://doi.org/10.1002/bjs.5719.
26. Farges O, Malassagne B, Flejou JF, Balzan S, Sauvanet A, Belghiti J. Risk of major liver resection in patients with underlying chronic liver disease: a reappraisal. Ann Surg. 1999;229(2):210–5.

27. Vauthey JN, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C, et al. Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. Surgery. 2000;127(5):512–9. https://doi.org/10.1067/msy.2000.105294.
28. Leung U, Simpson AL, Araujo RL, Gonen M, McAuliffe C, Miga MI, et al. Remnant growth rate after portal vein embolization is a good early predictor of post-hepatectomy liver failure. J Am Coll Surg. 2014;219(4):620–30. https://doi.org/10.1016/j.jamcollsurg.2014.04.022.
29. Farges O, Belghiti J, Kianmanesh R, Regimbeau JM, Santoro R, Vilgrain V, et al. Portal vein embolization before right hepatectomy: prospective clinical trial. Ann Surg. 2003;237(2):208–17. https://doi.org/10.1097/01.SLA.0000048447.16651.7B.
30. Ogata S, Belghiti J, Farges O, Varma D, Sibert A, Vilgrain V. Sequential arterial and portal vein embolizations before right hepatectomy in patients with cirrhosis and hepatocellular carcinoma. Br J Surg. 2006;93(9):1091–8. https://doi.org/10.1002/bjs.5341.
31. Yoo H, Kim JH, Ko GY, Kim KW, Gwon DI, Lee SG, et al. Sequential transcatheter arterial chemoembolization and portal vein embolization versus portal vein embolization only before major hepatectomy for patients with hepatocellular carcinoma. Ann Surg Oncol. 2011;18(5):1251–7. https://doi.org/10.1245/s10434-010-1423-3.
32. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg. 2012;255(3):405–14. https://doi.org/10.1097/SLA.0b013e31824856f5.
33. Vennarecci G, Laurenzi A, Levi Sandri GB, Busi Rizzi E, Cristofaro M, Montalbano M, et al. The ALPPS procedure for hepatocellular carcinoma. Eur J Surg Oncol. 2014;40(8):982–8. https://doi.org/10.1016/j.ejso.2014.04.002.
34. Chia DKA, Yeo Z, Loh SEK, Iyer SG, Madhavan K, Kow AWC. ALPPS for hepatocellular carcinoma is associated with decreased liver remnant growth. J Gastrointest Surg. 2018;22(6):973–80. https://doi.org/10.1007/s11605-018-3697-x.
35. Vennarecci G, Ferraro D, Tudisco A, Levi Sandri GB, Guglielmo N, Berardi G, et al. The ALPPS procedure: hepatocellular carcinoma as a main indication. An Italian single-center experience. Updat Surg. 2018;71:67–75. https://doi.org/10.1007/s13304-018-0596-3.
36. Ye JZ, Miao ZG, Wu FX, Zhao YN, Ye HH, Li LQ. Recurrence after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. Asian Pac J Cancer Prev. 2012;13(5):1771–7.
37. Fisher SB, Kneuertz PJ, Dodson RM, Patel SH, Maithel SK, Sarmiento JM, et al. A comparison of right posterior sectorectomy with formal right hepatectomy: a dual-institution study. HPB (Oxford). 2013;15(10):753–62. https://doi.org/10.1111/hpb.12126.
38. Yip VS, Poon RT, Chok KS, Chan AC, Dai WC, Tsang SH, et al. Comparison of survival outcomes between right posterior sectionectomy and right hepatectomy for hepatocellular carcinoma in cirrhotic liver: a single-centre experience. World J Surg. 2015;39(11):2764–70. https://doi.org/10.1007/s00268-015-3146-x.
39. Lee J, Lim DH, Kim S, Park SH, Park JO, Park YS, et al. Phase III trial comparing capecitabine plus cisplatin versus capecitabine plus cisplatin with concurrent capecitabine radiotherapy in completely resected gastric cancer with D2 lymph node dissection: the ARTIST trial. J Clin Oncol. 2012;30(3):268–73. https://doi.org/10.1200/JCO.2011.39.1953.
40. Chen X, Li B, He W, Wei YG, Du ZG, Jiang L. Mesohepatectomy versus extended hemihepatectomy for centrally located hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2014;13(3):264–70.
41. Starzl TE, Bell RH, Beart RW, Putnam CW. Hepatic trisegmentectomy and other liver resections. Surg Gynecol Obstet. 1975;141(3):429–37.
42. Fortner JG, Kim DK, Maclean BJ, Barrett MK, Iwatsuki S, Turnbull AD, et al. Major hepatic resection for neoplasia: personal experience in 108 patients. Ann Surg. 1978;188(3):363–71.
43. Schwartz SI. Right hepatic lobectomy. Am J Surg. 1984;148(5):668–73.
44. Liu CL, Fan ST, Lo CM, Tung-Ping Poon R, Wong J. Anterior approach for major right hepatic resection for large hepatocellular carcinoma. Ann Surg. 2000;232(1):25–31.

45. Beppu T, Imai K, Okuda K, Eguchi S, Kitahara K, Taniai N, et al. Anterior approach for right hepatectomy with hanging maneuver for hepatocellular carcinoma: a multi-institutional propensity score-matching study. J Hepatobiliary Pancreat Sci. 2017;24(3):127–36. https://doi.org/10.1002/jhbp.431.
46. Chan KM, Wang YC, Wu TH, Lee CF, Wu TJ, Chou HS, et al. The preference for anterior approach major hepatectomy: experience over 3 decades and a propensity score-matching analysis in right hepatectomy for hepatocellular carcinoma. Medicine (Baltimore). 2015;94(34):e1385. https://doi.org/10.1097/MD.0000000000001385.
47. Belghiti J, Guevara OA, Noun R, Saldinger PF, Kianmanesh R. Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. J Am Coll Surg. 2001;193(1):109–11.
48. Tang YH, Wen TF, Chen X. Resection margin in hepatectomy for hepatocellular carcinoma: a systematic review. Hepato-Gastroenterology. 2012;59(117):1393–7. https://doi.org/10.5754/hge10600.
49. Ikai I, Itai Y, Okita K, Omata M, Kojiro M, Kobayashi K, et al. Report of the 15th follow-up survey of primary liver cancer. Hepatol Res. 2004;28(1):21–9.
50. Schiffman SC, Woodall CE, Kooby DA, Martin RC, Staley CA, Egnatashvili V, et al. Factors associated with recurrence and survival following hepatectomy for large hepatocellular carcinoma: a multicenter analysis. J Surg Oncol. 2010;101(2):105–10. https://doi.org/10.1002/jso.21461.
51. Cha CH, Ruo L, Fong Y, Jarnagin WR, Shia J, Blumgart LH, et al. Resection of hepatocellular carcinoma in patients otherwise eligible for transplantation. Ann Surg. 2003;238(3):315–21; discussion 21–3. https://doi.org/10.1097/01.sla.0000086548.84705.ef.
52. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2. https://doi.org/10.1002/hep.24199.
53. Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. Gut. 2014;63(5):844–55. https://doi.org/10.1136/gutjnl-2013-306627.
54. Lu LC, Cheng AL, Poon RT. Recent advances in the prevention of hepatocellular carcinoma recurrence. Semin Liver Dis. 2014;34(4):427–34. https://doi.org/10.1055/s-0034-1394141.
55. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol. 2003;38(2):200–7.
56. Belghiti J, Fuks D. Liver resection and transplantation in hepatocellular carcinoma. Liver Cancer. 2012;1(2):71–82. https://doi.org/10.1159/000342403.
57. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med. 2008;359(19):1995–2004. https://doi.org/10.1056/NEJMoa0804525.
58. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. 2018;68(3):526–49. https://doi.org/10.1016/j.jhep.2017.09.016.
59. Subramanian J, Simon R. What should physicians look for in evaluating prognostic gene-expression signatures? Nat Rev Clin Oncol. 2010;7(6):327–34. https://doi.org/10.1038/nrclinonc.2010.60.
60. Berguer R, Rab GT, Abu-Ghaida H, Alarcon A, Chung J. A comparison of surgeons' posture during laparoscopic and open surgical procedures. Surg Endosc. 1997;11(2):139–42.
61. Cadiere GB, Himpens J, Germay O, Izizaw R, Degueldre M, Vandromme J, et al. Feasibility of robotic laparoscopic surgery: 146 cases. World J Surg. 2001;25(11):1467–77.
62. Martin RC, Scoggins CR, McMasters KM. Laparoscopic hepatic lobectomy: advantages of a minimally invasive approach. J Am Coll Surg. 2010;210(5):627–34,. 34-6. https://doi.org/10.1016/j.jamcollsurg.2009.12.022.

63. Cai XJ, Wang YF, Liang YL, Yu H, Liang X. Laparoscopic left hemihepatectomy: a safety and feasibility study of 19 cases. Surg Endosc. 2009;23(11):2556–62. https://doi.org/10.1007/s00464-009-0454-y.
64. Dagher I, Di Giuro G, Dubrez J, Lainas P, Smadja C, Franco D. Laparoscopic versus open right hepatectomy: a comparative study. Am J Surg. 2009;198(2):173–7. https://doi.org/10.1016/j.amjsurg.2008.09.015.
65. Abu Hilal M, Di Fabio F, Teng MJ, Lykoudis P, Primrose JN, Pearce NW. Single-centre comparative study of laparoscopic versus open right hepatectomy. J Gastrointest Surg. 2011;15(5):818–23. https://doi.org/10.1007/s11605-011-1468-z.
66. Takahara T, Wakabayashi G, Beppu T, Aihara A, Hasegawa K, Gotohda N, et al. Long-term and perioperative outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma with propensity score matching: a multi-institutional Japanese study. J Hepatobiliary Pancreat Sci. 2015;22(10):721–7. https://doi.org/10.1002/jhbp.276.
67. Tsung A, Geller DA, Sukato DC, Sabbaghian S, Tohme S, Steel J, et al. Robotic versus laparoscopic hepatectomy: a matched comparison. Ann Surg. 2014;259(3):549–55. https://doi.org/10.1097/SLA.0000000000000250.
68. Montalti R, Berardi G, Patriti A, Vivarelli M, Troisi RI. Outcomes of robotic vs laparoscopic hepatectomy: a systematic review and meta-analysis. World J Gastroenterol. 2015;21(27):8441–51. https://doi.org/10.3748/wjg.v21.i27.8441.
69. Vigano L, Laurent A, Tayar C, Tomatis M, Ponti A, Cherqui D. The learning curve in laparoscopic liver resection: improved feasibility and reproducibility. Ann Surg. 2009;250(5):772–82. https://doi.org/10.1097/SLA.0b013e3181bd93b2.
70. Kluger MD, Vigano L, Barroso R, Cherqui D. The learning curve in laparoscopic major liver resection. J Hepatobiliary Pancreat Sci. 2013;20(2):131–6. https://doi.org/10.1007/s00534-012-0571-1.
71. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34. https://doi.org/10.1016/S1470-2045(08)70285-7.
72. Bruix J, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, et al. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2015;16(13):1344–54. https://doi.org/10.1016/S1470-2045(15)00198-9.
73. von Marschall Z, Scholz A, Cramer T, Schafer G, Schirner M, Oberg K, et al. Effects of interferon alpha on vascular endothelial growth factor gene transcription and tumor angiogenesis. J Natl Cancer Inst. 2003;95(6):437–48.
74. Dinney CP, Bielenberg DR, Perrotte P, Reich R, Eve BY, Bucana CD, et al. Inhibition of basic fibroblast growth factor expression, angiogenesis, and growth of human bladder carcinoma in mice by systemic interferon-alpha administration. Cancer Res. 1998;58(4):808–14.
75. Wang L, Wu WZ, Sun HC, Wu XF, Qin LX, Liu YK, et al. Mechanism of interferon alpha on inhibition of metastasis and angiogenesis of hepatocellular carcinoma after curative resection in nude mice. J Gastrointest Surg. 2003;7(5):587–94.
76. Breitenstein S, Dimitroulis D, Petrowsky H, Puhan MA, Mullhaupt B, Clavien PA. Systematic review and meta-analysis of interferon after curative treatment of hepatocellular carcinoma in patients with viral hepatitis. Br J Surg. 2009;96(9):975–81. https://doi.org/10.1002/bjs.6731.
77. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. J Hepatol. 2010;52(6):889–94. https://doi.org/10.1016/j.jhep.2009.12.041.
78. Miyake Y, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. J Viral Hepat. 2010;17(4):287–92. https://doi.org/10.1111/j.1365-2893.2009.01181.x.

79. Zarrinpar A, Busuttil RW. Liver transplantation: past, present and future. Nat Rev Gastroenterol Hepatol. 2013;10(7):434–40. https://doi.org/10.1038/nrgastro.2013.88.
80. Philosophe B. Liver transplantation. In: Current surgical therapy. Philadelphia: Elsevier, Inc; 2017. p. 389–99.
81. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology. 2003;124(1):91–6. https://doi.org/10.1053/gast.2003.50016.
82. Hackl C, Schlitt HJ, Kirchner GI, Knoppke B, Loss M. Liver transplantation for malignancy: current treatment strategies and future perspectives. World J Gastroenterol. 2014;20(18):5331–44. https://doi.org/10.3748/wjg.v20.i18.5331.
83. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol. 2009;10(1):35–43. https://doi.org/10.1016/S1470-2045(08)70284-5.
84. Decaens T, Roudot-Thoraval F, Hadni-Bresson S, Meyer C, Gugenheim J, Durand F, et al. Impact of UCSF criteria according to pre- and post-OLT tumor features: analysis of 479 patients listed for HCC with a short waiting time. Liver Transpl. 2006;12(12):1761–9. https://doi.org/10.1002/lt.20884.
85. Unek T, Karademir S, Arslan NC, Egeli T, Atasoy G, Sagol O, et al. Comparison of Milan and UCSF criteria for liver transplantation to treat hepatocellular carcinoma. World J Gastroenterol. 2011;17(37):4206–12. https://doi.org/10.3748/wjg.v17.i37.4206.
86. Koschny R, Schmidt J, Ganten TM. Beyond Milan criteria—chances and risks of expanding transplantation criteria for HCC patients with liver cirrhosis. Clin Transpl. 2009;23(Suppl 21):49–60. https://doi.org/10.1111/j.1399-0012.2009.01110.x.
87. Tayar C, Kluger MD, Laurent A, Cherqui D. Optimizing outflow in piggyback liver transplantation without caval occlusion: the three-vein technique. Liver Transpl. 2011;17(1):88–92. https://doi.org/10.1002/lt.22201.
88. Grewal HP, Shokouh-Amiri MH, Vera S, Stratta R, Bagous W, Gaber AO. Surgical technique for right lobe adult living donor liver transplantation without venovenous bypass or portocaval shunting and with duct-to-duct biliary reconstruction. Ann Surg. 2001;233(4):502–8.
89. Grat M, Kornasiewicz O, Lewandowski Z, Skalski M, Zieniewicz K, Paczek L, et al. The impact of surgical technique on the results of liver transplantation in patients with hepatocellular carcinoma. Ann Transplant. 2013;18:448–59. https://doi.org/10.12659/AOT.884005.
90. Reichman TW, Katchman H, Tanaka T, Greig PD, McGilvray ID, Cattral MS, et al. Living donor versus deceased donor liver transplantation: a surgeon-matched comparison of recipient morbidity and outcomes. Transpl Int. 2013;26(8):780–7. https://doi.org/10.1111/tri.12127.
91. Sharma S, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. Liver Transpl. 2008;14(6):759–69. https://doi.org/10.1002/lt.21509.
92. Lin M, Crawford M, Fisher J, Hitos K, Verran D. Hepatic artery thrombosis and intraoperative hepatic artery flow rates in adult orthotopic liver transplantation. ANZ J Surg. 2002;72(11):798–800.
93. Pratschke S, Meimarakis G, Mayr S, Graeb C, Rentsch M, Zachoval R, et al. Arterial blood flow predicts graft survival in liver transplant patients. Liver Transpl. 2011;17(4):436–45. https://doi.org/10.1002/lt.22248.
94. Shen JY, Li C, Wen TF, Yan LN, Li B, Wang WT, et al. Liver transplantation versus surgical resection for HCC meeting the Milan criteria: a propensity score analysis. Medicine (Baltimore). 2016;95(52):e5756. https://doi.org/10.1097/MD.0000000000005756.
95. Shen JY, Li C, Wen TF, Yan LN, Li B, Wang WT, et al. Transplantation versus hepatectomy for HCC beyond the Milan criteria: a propensity score analysis. Int J Surg. 2017;44:33–42. https://doi.org/10.1016/j.ijsu.2017.05.034.

96. Proneth A, Zeman F, Schlitt HJ, Schnitzbauer AA. Is resection or transplantation the ideal treatment in patients with hepatocellular carcinoma in cirrhosis if both are possible? A systematic review and metaanalysis. Ann Surg Oncol. 2014;21(9):3096–107. https://doi.org/10.1245/s10434-014-3808-1.
97. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Difference in tumor invasiveness in cirrhotic patients with hepatocellular carcinoma fulfilling the Milan criteria treated by resection and transplantation: impact on long-term survival. Ann Surg. 2007;245(1):51–8. https://doi.org/10.1097/01.sla.0000225255.01668.65.
98. Koniaris LG, Levi DM, Pedroso FE, Franceschi D, Tzakis AG, Santamaria-Barria JA, et al. Is surgical resection superior to transplantation in the treatment of hepatocellular carcinoma? Ann Surg. 2011;254(3):527–37; discussion 37–8. https://doi.org/10.1097/SLA.0b013e31822ca66f.
99. Kim RD, Hemming AW. Hepatocellular carcinoma: resection or transplantation. J Gastrointest Surg. 2009;13(6):1023–5. https://doi.org/10.1007/s11605-008-0784-4.
100. Lee SY, Sadot E, Chou JF, Gonen M, Kingham TP, Allen PJ, et al. Central hepatectomy versus extended hepatectomy for liver malignancy: a matched cohort comparison. HPB (Oxford). 2015;17(11):1025–32. https://doi.org/10.1111/hpb.12507.

Chapter 9
Interventional Radiologic Therapies for Hepatocellular Carcinoma: From Where We Began to Where We Are Going

Patrick D. Sutphin, Daniel Lamus, Sanjeeva P. Kalva, Junjie Li, and Ian R. Corbin

Introduction: Liver and Tumor Blood Supply

The liver is the recipient of a dual blood supply. The portal vein transports venous blood from the gastrointestinal tract and spleen to the liver and accounts for approximately 75% of the blood flow to the liver and 50% of the oxygen content. The arterial supply to the liver from the hepatic artery provides 25% of the blood supply and the remaining 50% of the oxygen. This relationship of the two blood supplies to discrete components of the liver inspired the investigation of the role of the two blood supplies in pathologic processes. Wright et al. examined liver necropsy specimens from 15 patients with metastatic liver disease following the injection of gelatin stained with either carmine (red) into the portal vein or Berlin blue into the hepatic

P. D. Sutphin · D. Lamus
Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

S. P. Kalva
Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Department of Radiology, Massachusetts General Hospital, Boston, MA, USA

J. Li
Advanced Imaging Research Center, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

I. R. Corbin (✉)
Advanced Imaging Research Center, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Department of Internal Medicine, Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA
e-mail: ian.corbin@utsouthwestern.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_9

artery. The tumor type was variable. In all cases, with the exception of microscopic disease, the tumors stained blue consistent with a hepatic arterial supply (Fig. 9.1). Histologic examination demonstrated that the branching arteries, arterioles, and capillaries could be traced back to branches of the hepatic artery [1].

Similarly, in the exploration of the hepatic circulation in rabbits, Breedis et al. demonstrated that injection of the portal vein with India ink resulted in intense staining of the hepatic parenchyma, whereas hepatic tumors failed to stain. In contradistinction, tumor tissue and liver parenchyma stained with India ink following injection of the hepatic artery [2]. These findings suggested that hepatic tumors were supplied predominantly, or perhaps exclusively by the hepatic artery (Fig. 9.2).

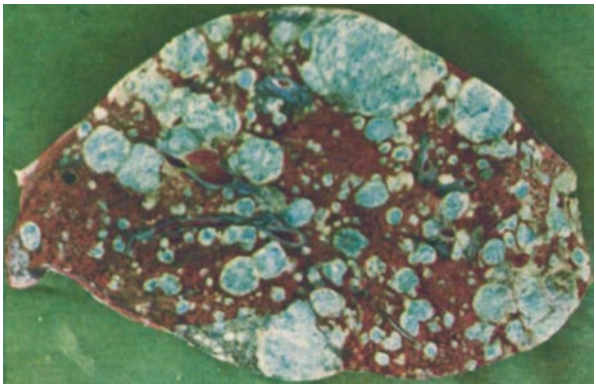

Fig. 9.1 Section of human liver containing multiple metastatic lesions. The liver was excised from human cadaver, and the hepatic artery and portal vein was cannulated and perfused with solutions of blue and red gelatin, respectively. Note all the metastatic lesions were stained with the blue gelatin, while the surrounding liver parenchyma was stained with red gelatin

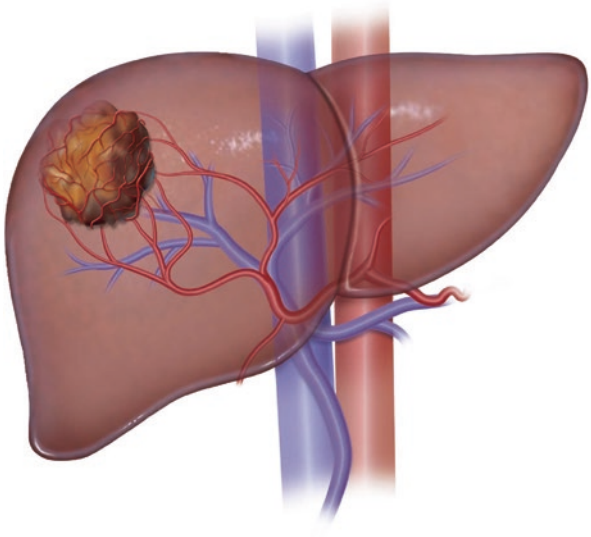

Fig. 9.2 Diagram showing the blood supply to the liver and hepatic tumor. The tumor derives 95% of its blood supply from the hepatic artery. Normal liver parenchyma receives 75% of its blood supply from the portal vein and the remaining 25% from the hepatic artery

The study was extended to include metastatic human disease, and the results reported in 11 livers obtained at autopsy confirmed the hepatic arterial supply to metastatic liver disease through injection experiments and histologic quantification of arterial versus portal venous vessels [2].

Physiologic Targeting Through the Hepatic Artery

Hepatic Artery Ligation

Interruption of the hepatic arterial supply to hepatic tumors was proposed as early as 1952 [3]. Breedis et al. reported attempting hepatic artery ligation in a small series of rabbits with VX2 carcinoma without evidence of tumor regression. Details and data from the small series were not provided. It was noted that the failure of regression may be secondary to the rich collateral supply to the liver so that the arterial supply to the tumors was not completely eliminated [2]. Nilsson and Zettergren later examined tumor vascular supply in an induced model of primary hepatic malignancy in rats. After chemical induction of cirrhosis with 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB), the rats developed hepatocellular carcinoma, cholangiocarcinoma, and mixed hepatocellular and cholangiocellular carcinomas. Vascular supply to the tumors was examined with x-ray micrography following infusion of 30% barium sulfate into either the hepatic artery or portal vein after euthanasia. The authors concluded that cholangiocarcinoma was supplied exclusively by the hepatic artery and that hepatocellular carcinoma is supplied by the hepatic artery, but it likely receives a contribution from the portal vein as well [4]. In the same rat model of induced liver cancer, Nilsson and Zettergren ligated the hepatic artery at the hilus in 19 rats and observed necrosis of tumors with some demonstrating reduction in size and no viable tumor on microscopic examination [5].

Clinical demonstration of the benefit of hepatic artery ligation was reported in 1964 in a patient with gastric carcinoma with liver metastasis. During a surgical procedure, the hepatic artery was accidently obstructed. The patient died 30 h post procedure, and autopsy revealed severe necrosis of the tumor with only slight damage evident in the surrounding liver [6]. Gelin et al. demonstrated a 90% reduction in blood flow to tumor tissue following hepatic artery ligation in three patients, compared to a 35–40% reduction in normal hepatic parenchyma [7]. Subsequent investigation of hepatic dearterialization in 27 patients did not provide survival benefit, but it was acknowledged to result in necrosis in the majority of tumor cells. It was concluded that the hepatic dearterialization should not be a stand-alone procedure but combined with other methods of treatment [8]. The failure of hepatic dearterialization to result in clinically meaningful benefit is secondary to the rich hepatic arterial collateralization. Angiographic studies of ten patients following hepatic artery ligation and dearterialization demonstrated the rapid development of arterial collateralization as early as 4 days post procedure, the earliest time examined (Fig. 9.3) [9].

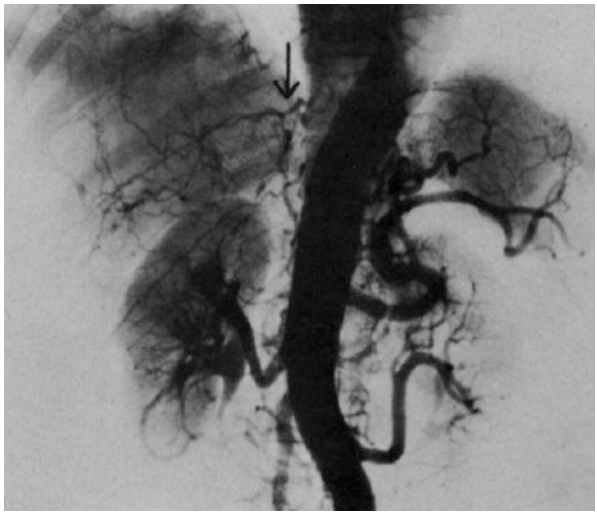

Fig. 9.3 Aortography 40 days after hepatic artery obstruction. At least two of the intercostal arteries (arrow) are widened and give collateral vessels to the liver

The development of techniques to directly catheterize branches of the aorta presented an opportunity to more directly administer antineoplastic agents or radioactivity directly to tumoral tissue. In 1951, Bierman described the experience of catheterizing the celiac and hepatic artery in 50 patients, most of whom had neoplastic involvement of the liver. In addition to describing the technique for hepatic catheterization, many of the angiographic hallmarks of tumor tissue were also reported including the increased vascular outlines of the tumor with small bizarre and disorderly branching patterns arising from disproportionately large parent arteries. It was also recognized that in addition to the diagnostic potential of hepatic angiography for liver metastases, hepatic artery catheterization also provided a direct conduit for therapeutic delivery [10, 11].

Hepatic Artery Infusion

Long-term hepatic artery infusion was evaluated in 28 patients with either primary or secondary liver cancer via transbrachial artery approach by Clarkson et al. in the early 1960s. Successful hepatic artery cannulation was achieved in 23 out of 28 patients with long-term administration of antimetabolic agents (e.g., 5-fluorouracil, methotrexate). Significant regression was reported in 9 of 16 patients, but it was noted to be brief, and it was ultimately concluded that the procedure may be of doubtful clinical benefit [12].

Transarterial Embolization

Chuang and Wallace sought to address the rapid arterial collateralization that followed hepatic ligation with both proximal and distal embolization of the hepatic artery. Forty-seven patients with primary or secondary liver cancer were examined across 72 embolization procedures from 1972 to 1979. Patients were embolized with a combination of gelatin sponge to the peripheral hepatic artery bed and proximal embolization with a stainless steel coil. The majority of patients had prior systemic or intra-arterial infusion of chemotherapy, and when chemotherapy failed hepatic artery embolization was the subsequent treatment option. Patients in the series had a median survival of 11.5 months from the embolization. The authors compared median survival times from the literature and from their own institution, leading the authors to conclude that this is an effective treatment for hepatic neoplasms [13].

Transarterial Chemoembolization

Kato et al. formally introduced the concept of chemoembolization with the intra-arterial delivery of mitomycin C encapsulated in ethylcellulose microparticles (225 μm) to treat a variety of tumor types at different sites. The concept was that the embolic agent would add ischemia and prolong transit through the arterial system improving localized drug delivery [14]. Though the patients demonstrated a high rate of response, the data did not suggest survival benefit in patients with several metastases but did often provide symptomatic relief. Chemoembolization was further studied in 120 patients with unresectable hepatoma. The authors mixed either mitomycin C or Adriamycin with gelatin sponge and contrast to embolize hepatic tumors. Objective tumor response was observed in 75% of cases, and the 1-year survival was 44% which was greater than that observed with surgery at that time 28% [15].

Nakamura et al. compared the chemoembolization with gelatin sponge in 104 patients to 100 patients treated with doxorubicin mixed with iodized oil infused intra-arterially followed by gelatin sponge embolization [16]. Compared with intra-arterial doxorubicin alone, serum concentrations of doxorubicin were significantly lower in patients treated with the in-oil emulsion. The authors compared the cumulative survival of 100 patients treated with iodized oil chemoembolization to a historical control of 104 patients treated with gelatin sponge chemoembolization with the suggestion of a survival benefit. A comparison of intra-arterial delivery of a doxorubicin-iodized emulsion with or without gelatin sponge embolization demonstrated significantly higher rates of complete tumor necrosis with the

inclusion of gelatin sponge embolization, 83% versus 13% [17]. No comparison was made between iodized oil and gelatin sponge embolization with and without doxorubicin.

Randomized Controlled Trials

Early studies in the use of transarterial embolization and transarterial chemoembolization compared the results across multiple tumor types, and the results were generally compared to historical controls. It was not until the late 1980s that randomized control studies were performed. In one of the early randomized controlled trials, Lin et al. randomized patients into three groups with 21 patients each. Group 1 patients had multiple rounds of hepatic artery embolization with polyvinyl alcohol particles and Gelfoam powder or cubes. Group 2 had a single HAE followed by monthly chemotherapy with 5-fluorouracil, and Group 3 was treated with 5-fluorouracil only. The results of the study found a survival benefit of multiple HAE over 5-fluorouracil alone [18].

Pelletier et al. randomized 42 patients to either be treated with Gelfoam chemoembolization or symptomatic treatment [19]. No significant difference in survival was noted between the two patient groups. A subsequent study by Pelletier et al. that randomized patients to lipiodol chemoembolization versus symptom control also failed to demonstrate a survival benefit for chemoembolization [20]. The lack of benefit has been attributed in part to broad selection criteria with patients treated up to age 80, with patients treated with bilirubin up to 2.8 mg/dL, and greater fraction of patients with performance status of 1 or greater.

Bruix et al. designed a randomized controlled trial to compare transarterial embolization with gelatin sponge cubes and proximal embolization with a steel coil when feasible (40 patients) to symptomatic control (40 patients). Though 55% of the patients in the TAE group demonstrated a partial response, no survival benefit was found between the TAE group and the symptom control group [21].

It was not until May 2002 that a pair of randomized controlled trials demonstrated a survival benefit of TACE relative to symptomatic control. In an asian randomized controlled trial in Okuda stage I/II HCC patients, Lo et al. demonstrated a survival benefit in patients with unresectable HCC treated with lipiodol chemoembolization with cisplatin compared to symptomatic treatment (Fig. 9.4a) [22]. In this study, 80% of the patients were seropositive for hepatitis B. The cohort of patients that was randomized to chemoembolization had a 57%, 31%, and 26% survival at 12, 24, and 36 months compared to a 32%, 11%, and 3% survival in patients that had symptomatic control. Similarly, 112 patients in a European cohort were randomly assigned to one of the three treatment arms (Fig. 9.4b) [23]. Treatment group 1 underwent transarterial embolization with gelatin sponge, group 2 underwent transarterial chemoembolization with gelatin sponge and doxorubicin with lipiodol, and group 3 received symptomatic treatment. The survival benefit observed in the study has been partially attributed to the restrictive selection criteria with an effort to exclude patients with factors that may lead to treatment intolerance

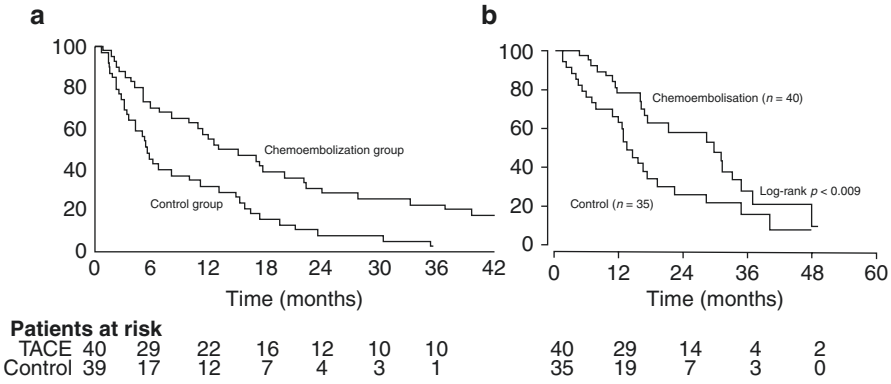

Fig. 9.4 Kaplan-Meier survival plots from randomized controlled trials to assess efficacy of chemoembolization. (**a**) Survival plot from a randomized controlled trial by Lo et al. to assess the efficacy of transarterial lipiodol chemoembolization with cisplatin in patients with unresectable HCC. (**b**) Survival plot from a randomized controlled trial to assess the efficacy of lipiodol chemoembolization with doxorubicin in patients with unresectable HCC. Labels have been modified for readability

or failure. The survival probability at 1 and 2 years with chemoembolization was 82% and 63% which was significantly higher relative to the symptomatic control group which had a 63% and 27% at 1 and 2 years. Embolization with Gelfoam had a survival probability of 75% and 50% at 1 and 2 years, which was not statistically different than either the chemoembolization group or symptomatic control group. It should be noted that the study was terminated early after the ninth sequential inspection revealed a survival benefit in patients treated with TACE relative to conservative treatment. The early termination of the study potentially underestimates the survival benefit of transarterial embolization relative to conservative management [23, 24].

Since the above trials TACE has become the standardized treatment for intermediate stage HCC. Additional developments in chemoembolization include the introduction of drug-eluting beads into the chemoembolization regimen. DC Beads were introduced in 2004 and represent an embolic drug delivery system [25]. The beads are biocompatible nonresorbable polyvinyl alcohol hydrogel beads which can be loaded with doxorubicin (Fig. 9.5). Preclinical and early clinical studies demonstrated embolization with DC Beads resulted in higher intratumoral doxorubicin concentrations and less systemic exposure relative to lipiodol TACE. Doxorubicin-eluting bead TACE (DEB-TACE) was directly compared to conventional TACE (cTACE) in a phase II randomized control multicenter trial (PRECISION V). Two hundred twelve patients were randomized to either receive cTACE or DEB-TACE. DEB-TACE was performed with one vial of 300–500 μm beads followed by one vial of 500–700 μm beads with 150 mg doxorubicin delivered per procedure. Tumor response was evaluated at 6 months according to the European Association for the Study of the Liver (EASL) criteria. Though there was a slight trend toward improved response with DEB-TACE, superiority of DEB-TACE over cTACE was not demonstrated statistically. cTACE had a complete response, objective response,

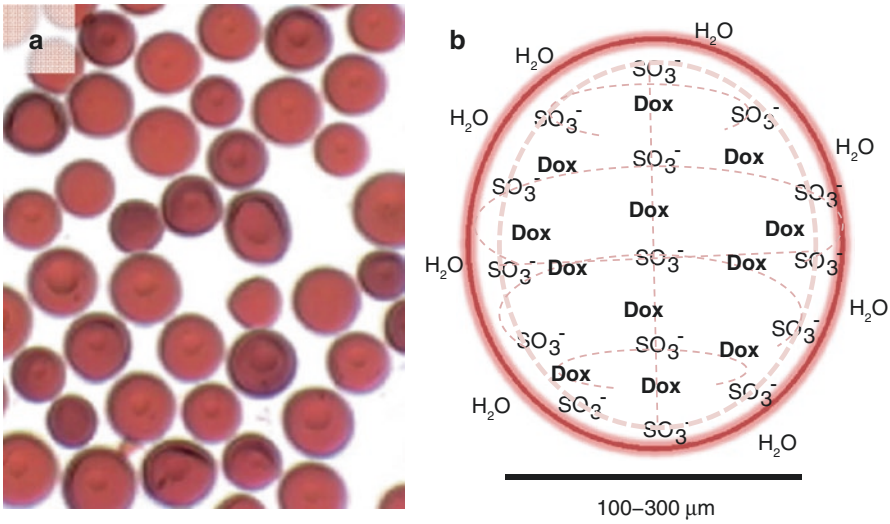

Fig. 9.5 Representative drug-eluting bead. (**a**) Photomicrograph of doxorubicin-loaded DC Beads® and (**b**) schematic of DC Bead microsphere. DC Bead microspheres (100–300 µm in diameter) consist of a polyvinyl alcohol hydrogel modified with sulfonate groups. The presence of the anionic sulfonate group enables the sequestering of positively charged drugs, such as doxorubicin, through ionic (Coulomb charge) interactions. The drug is slowly released from the beads in the targeted site

and disease control rate of 22%, 44%, and 52%, respectively, compared to 27%, 52%, and 63%, respectively, for DEB-TACE [26]. The study found improved tolerability of DEB-TACE with fewer adverse effects relative to cTACE. A subsequent randomized controlled study of 177 patients randomized to either DEB-TACE or cTACE found no significant difference in local or overall tumor response and median time to progression of 9 months in both arms. In this study DEB-TACE was performed with 100–300 µm beads loaded with 50 mg doxorubicin per vial, and cTACE was performed with a maximum dose of 75 mg epirubicin. The incidence of adverse events was also similar between the two groups with the exception of less post-procedural abdominal pain in the DEB-TACE group. The overall 1- and 2-year survival rates for cTACE were 83.5% and 55.4% compared to 86.2% and 56.8% for DEB-TACE [27].

Radioembolization

Early Evaluation

In addition to chemotherapy, arterial catheterization provides an avenue for the delivery of radioactive agents directly to tumor tissue [28]. In the context of radiotherapy, infusional therapy is less favorable due to the risks of systemic

accumulation of radioisotopes and the potential of excretion of radioactivity. Thus, the advantage of lodging the therapeutic radioisotope in the precapillary bed of tumor tissue was recognized early. Initial studies of intravascular delivery of radioactivity were performed for the treatment of lung cancer. Muller and Rossier injected particulate radioactive gold (Au 198) on charcoal intravenously with deposition in the lungs for the treatment of lung cancer [29]. Later studies evaluated the use of radioactive particles in both animals and human for the treatment of cancer.

Grady et al. examined the use of intravascular injection of large yttrium-90 oxide particles in rabbits, dogs, and subsequently human patients [30]. Intravascular injection of yttrium-90 oxide particles was used to treat 12 patients with lung and pelvic cancer and 5 patients with primary or secondary liver cancer [30]. Other embolic agents used in other early studies with intra-arterial radiation therapy include ceramic microspheres manufactured by the Minnesota Mining and Manufacturing Company (3M) labeled with yttrium-90 and a resin microsphere labeled with phosphorus-32 [31–35].

One of the early complications of the administration of the ceramic microspheres were the development of numerous small petechial-like irradiation reaction sites due to the gravity-dependent posterior deposition of microspheres due to the heavy density of the particles [32]. Delivering the microspheres in the prone position resolved this complication. The difficulty with suspension of the radioactive particles in solution for intra-arterial delivery with either yttrium oxide or ceramic microspheres led to the transition to the resin-based microsphere [36].

Yttrium-90

Yttrium-90 has many characteristics that make it a suitable radionuclide for the treatment of cancer. Y90 is a high-energy (maximum 2.27 MeV with mean of 0.937 MeV) pure beta emitter as it decays to zirconium 90. There is limited tissue penetration of the high-energy beta ray with an average distance of 2.5 mm and maximum of 11 mm [37, 38]. The limited tissue penetration provides focal radiation exposure without adverse effect to the bone marrow or the need for isolation of the patient (Fig. 9.6). The half-life is short at 2.67 days, and greater than 95% of the radiation is delivered within 2 weeks [35, 36]. In addition, the Bremsstrahlung gamma ray produced secondarily by beta activity is sufficient for both radiation survey with a Geiger-Muller counter and imaging of microsphere deposition.

Current State

A shift in the algorithm for the treatment of intermediate stage HCC may be underway. Recently, Salem et al. provided the rationale for the institutional decision to transition to radioembolization with Y90 as the primary treatment for HCC based on

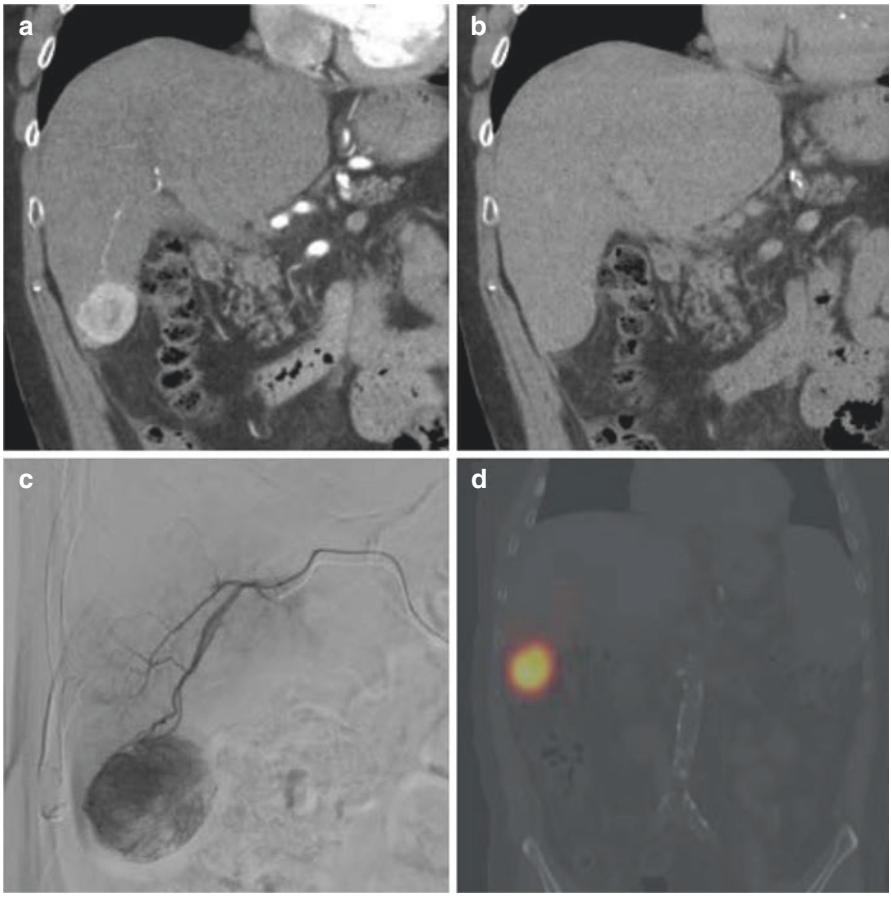

Fig. 9.6 A 73-year-old man with alcoholic cirrhosis and segment VI hepatocellular carcinoma treated with Y90 radiation segmentectomy. (**a**) Arterial phase and (**b**) delayed phase coronal images from a four-phase CT demonstrate a 3.6 cm segment VI LiRads-5 lesion with arterial enhancement and delayed washout and pseudocapsule. (**c**) Segmental angiogram just prior to delivery of Y90 glass beads. (**d**) SPECT-CT from technetium-99m macroaggregated albumin (99mTc MAA) from planning study demonstrating preferential delivery of arterially administered 99mTc MAA to tumor tissue relative to adjacent liver

a 15-year experience with 1000 patients [39]. Though no survival benefit has been demonstrated with Y90 in intermediate stage HCC, Y90 does have some advantages over TACE. A recent randomized control phase II study compared cTACE with Y90 radioembolization in the treatment of HCC. Radioembolization demonstrated improved time to progression, the study's primary endpoint. Radioembolization patients had a median TTP of >26 months compared to 6.8 months in cTACE patients. Despite the differences in TTP, no survival benefit was demonstrated [40]. Similar results were obtained in a retrospective propensity-matched study comparing segmental radioembolization to segmental TACE with the findings of increased progression-free survival with radioembolization [41].

In addition to increased time to progression, radioembolization is associated with improved quality of life relative to TACE. Retrospective studies have found a reduced rate of post-embolization syndrome and post-procedure hospitalization. A prospective study assessed the quality of life at 2 and 4 weeks posttreatment. Despite more advanced disease, radioembolization improved quality of life related to functional well-being and social well-being relative to TACE patients. In the phase II randomized controlled trial, delayed toxicities occurred in 3 of 21 cTACE patients and in 4 of 24 patients. It was reported that three of the four patients with delayed toxicity following radioembolization were related to ascites. Ascites has been found to be associated with reduced quality of life in cirrhotic patients [42–44].

Finally, another advantage of radioembolization over TACE is the ability to treat patients with portal vein invasion and occlusion. It was recognized early in the treatment of patients with HCC with embolization techniques that patients with portal vein invasion were at higher risk for complications and death. Yamada et al. demonstrated that five of nine patients treated with transarterial embolization with gelatin sponge mixed with chemotherapy died within 30 days. In three of the cases, extensive necrosis was observed in the tumor as well as the surrounding liver parenchyma [15]. The smaller particle size in radioembolization is used primarily for the intratumoral distribution of the radionuclide and results in less arterial occlusion and less ischemia than with TACE. The lack of embolic effect and induction of ischemia reduces the risk of hepatic necrosis in patients with compromised portal flow broadening the spectrum of treatable disease.

Innovations in Transarterial Therapies

There has been a steady increase over the last several years in the use of transarterial locoregional treatments for HCC. Despite the increasing interest and popularity of radioembolization, TACE remains the most common palliative treatment for HCC [45, 46]. Population-based data demonstrate that more patients with HCC are treated with TACE than all other HCC therapies combined [45, 46]. Unfortunately, progress toward improving survival or palliation with conventional transarterial approaches has been slow. To overcome this incremental pace forward, innovative strategies utilizing novel delivery systems should be employed. Nano- and microscale drug carriers have received considerable attention in recent years as they promise to improve the bioavailability, solubility, delivery efficiency, and therapeutic effectiveness of anticancer agents. To date, there are numerous preclinical publications in the field of interventional oncology, many of which describe the transarterial delivery of conventional chemotherapies (e.g., doxorubicin) via polymer or liposome-based carriers. These approaches, however, are more or less extensions of current treatment paradigms. Biomimetic or bioinspired vehicles represent a truly novel class of nanocarriers that offer a highly innovative and “out-of-the-box” approach to nanomedicine. Bioinspiration and biomimicry describe technologies that exploit or recapitulate biological materials not only from the standpoint of chemistry and structure but also in terms of their biological characteristics and functions [47]. Nature serves

as the perfect source of inspiration for designing biomaterials and nanotechnologies that are able to overcome the many physical and biological barriers that impede successful drug delivery to tumors [48, 49]. Whether these technologies exploit natural molecular assemblies, organisms, or cells, this innovative approach enables unprecedented access, entry, and delivery of therapeutic payloads into cells via seamless natural biological processes [47]. In this section we will describe various preclinical bioinspired nanoparticle strategies that have been used in conjunction with transarterial delivery for the treatment of HCC; these include immune cell, viral, and lipoprotein-like carriers.

Natural Killer Cell-Based Therapy

Immune-cell-like therapies have received much attention in cancer research, due to the intrinsic tumoricidal and cytotoxic capacity of these cells. Human natural killer (NK) cells are cytotoxic lymphocytes that play a critical role in the innate immune system and tumor immune-surveillance [50]. Not requiring prior activation or antigen priming these cells are able to identify and kill their target tumor cells in the absence of major histocompatibility complex (MHC) presentation [51, 52]. Furthermore, NK cells are known to function as effectors of innate immunity, their secretion of cytokines, such as IFNγ and TNFα, can activate macrophage, dendritic cells and neutrophils, which subsequently enables antigen-specific T and B cell responses. More recently NK cells have also been implicated in playing a role in the adaptive immune response [52]. For many of the aforementioned reasons NK cell-based adoptive transfer immunotherapy (ATI) holds great promise for the treatment of solid tumors, including HCC. The clinical experience with NK-ATI, however, has only produced modest results for cancer patients [53, 54]. Two hurdles that currently limit the efficacy of NK-ATI include: (1) inadequate homing efficiency of NKs to the targeted tumors and (2) lack of well-established noninvasive tools for predicting the NK-ATI response. To address these issues the group of Larson and Zhang studied the transarterial delivery of superparamagnetic iron oxide (SPIO) labeled NK cells in a rat model of HCC [55, 56]. The SPIO-label allowed for ***in vivo*** noninvasive tomographic tracking and quantitation of NK cells by MRI, while the local delivery through the hepatic artery ensured adequate targeting of the HCC lesion. These studies showed that as early as 24 or 48 h after local transarterial delivery of SPIO-labeled NK cells, MRI was able to track NK cell homing to the targeted tumor [56]. An increase in the MRI R2∗ relaxivity signal within the tumor at 24 h was indicative of SPIO-NK cell accumulation within the tumor (see Fig. 9.7a) [56]. Histological evaluations confirmed these findings as high immunostaining for CD56 expression, a marker for NK cells, was also found in the HCC lesions. The early uptake of SPIO-NK cells correlated with the therapeutic response of marked tumor growth inhibition measured at 8 days post treatment (Fig. 9.7b) [56]. Collectively, these findings demonstrate the utility of transarterial NK-ATI for HCC therapy. Furthermore, serial MRI monitoring of NK

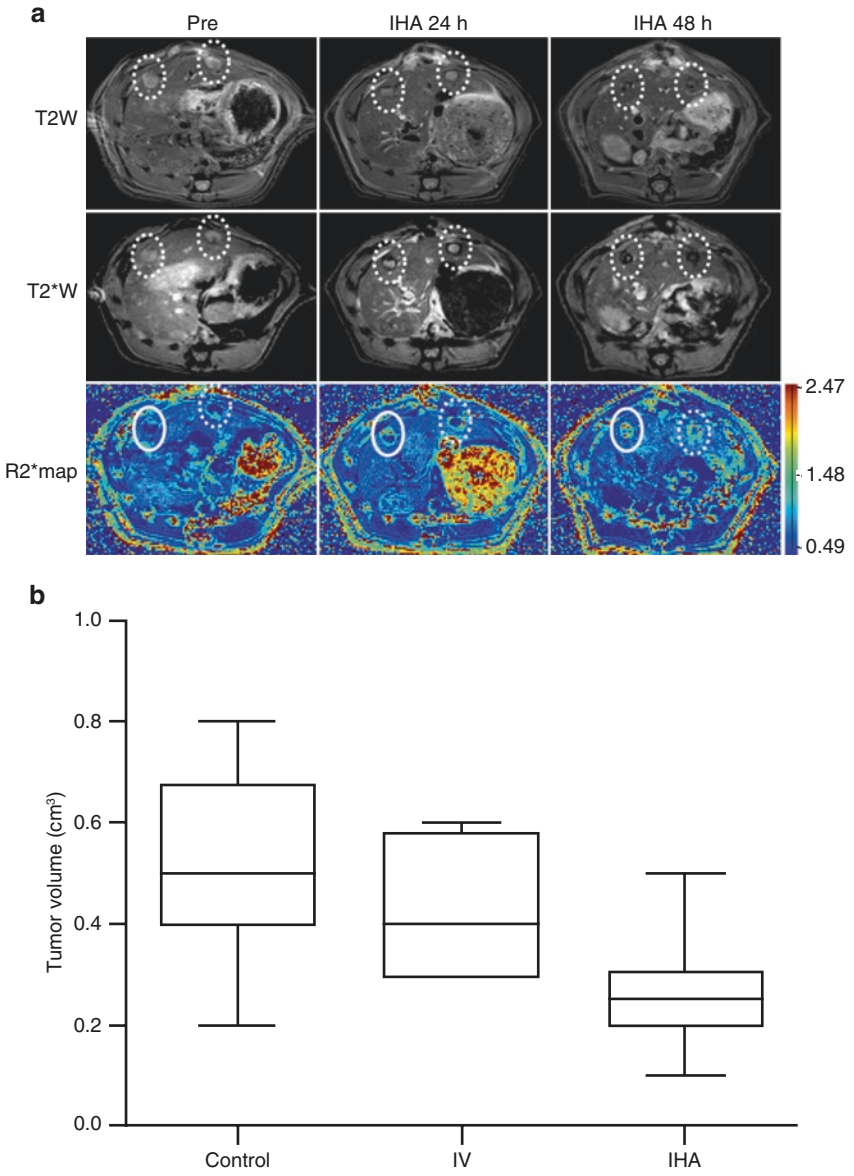

Fig. 9.7 Quantitative MRI of labeled NK cell biodistribution in the targeted tumors. (**a**) Representative T2W and T2∗W images and R2∗ maps at pre- and postinfusion intervals (24 h and 48 h). T2W images at preinfusion and postinfusion 24 h and 48 h are on the top row. T2∗W images at preinfusion and postinfusion are on the middle row. Quantitative R2∗ maps at preinfusion and postinfusion 24 h and 48 h are on the bottom row. Circles indicate tumor locations. (**b**) Graph shows the changes of tumor volumes at 8 days after IHA infusion. Effective inhibition of tumor growth can be observed 8 days after IHA infusion (control group vs. IHA group, $P < 0.001$; IHA group vs. IV group, $P = 0.001$); no significant difference in therapeutic response was observed between control group and IV group 8 days after infusion ($P = 0.196$)

migration to targeted tumors is feasible and early post treatment imaging of tumor R2∗ may serve as an important biomarker for prediction of longitudinal treatment response.

Virus-Based Therapies

Viruses naturally exhibit several characteristics that are of interest for drug delivery due to their intrinsic ability to avoid immune system recognition and gain entry into cells. In this capacity viruses can serve as viral vectors for gene delivery or as tumor-targeted replication-competent viruses (i.e., oncolytic agents). In this section we will review transarterial applications of vesicular stomatitis virus (oncolytic virus) and adenovirus (gene vector) for the treatment of HCC.

Vesicular Stomatitis Virus: Oncolytic Virus

Vesicular stomatitis virus (VSV) is an enveloped, negative-strand RNA virus (Rhabdoviridae family) that infects a wide variety of mammalian cells. Typically, this virus does not persist in normal cells due to the induction of interferon and the robust antiviral response. Conversely, in many tumors the IFN-responsive antiviral pathways are defective; hence this virus selectively replicates at high rates in tumor cells [57]. As VSV replicates, it destroys the infected tumor cell by oncolysis, releasing new infectious virus particles that go on to destroy neighboring cancer cells. Compared with other replication-competent oncolytic vectors, VSV is particularly appealing due to its rapid replication rate (8–10 h in tumor cells) [58]. Such high rates of viral replication enable rapid antitumor effects within hours of injection, and significant tumor destruction could occur before the initiation of potentially neutralizing antiviral immune responses. Studies from the Woo lab demonstrated that hepatic arterial infusion of recombinant VSV at the maximum tolerated dose (MTD) in tumor-bearing rats resulted in efficient viral transduction of multifocal HCC lesions, tumor-selective viral replication, and extensive oncolysis (Fig. 9.8a, b) [58]. Importantly, no significant vector-associated hepatotoxicity was noted. Finally, survival of vector-treated rats was substantially prolonged over that of animals in the control treatment group ($p < 0.028$) (Fig. 9.8c) [58]. The therapeutic window of VSV administered at MTD, however, is quite narrow as neurotoxicity and acute lethal hepatotoxicity are seen at doses above MTD [59]. To improve the safety of the VSV-based oncolytic virotherapy, this group recently evaluated the prophylactic administration of IFN-α (equivalent to clinical dose prescribed to viral hepatitis patients) in tumor-bearing rats before transarterial VSV therapy. The investigators concluded that IFN-α pretreatment was able to quench any lethal systemic proinflammatory responses triggered by high loads of administered VSV. This intervention increased the VSV MTD by ½ log unit [59]. Intratumoral VSV replication was not attenuated by exogenous administration of IFN-α, and

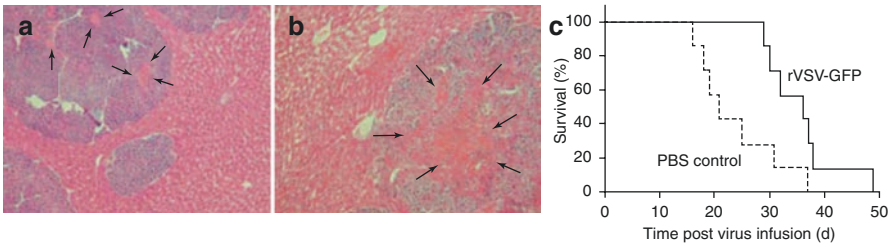

Fig. 9.8 Histopathology of liver sections of tumor-bearing animals after hepatic arterial infusion of VSV. Representative H&E sections of the liver (**a**) before and (**b**) 1 day after virus infusion. Arrows indicate necrotic areas. (**c**) Kaplan-Meier survival curve of rats with multifocal HCC after hepatic arterial infusion of VSV versus PBS. Animals with multifocal HCC were infused with 1.3 × 107 pfu rVSV-GFP (solid line) or PBS control (dashed line) via the hepatic artery on day 0 and followed for survival. The survival advantage for VSV-administered animals was statistically significant compared with PBS control animals (P = 0.028, log-rank test). The results were combined from two consecutive sets of animals with stratification

tumor response and survival advantages in the VSV-treated rats greatly surpassed that of untreated controls [59]. These findings are particularly pertinent as human IFN-α is currently in clinical use, thus its prophylactic application could be considered in future translational protocols of VSV-mediated oncolytic therapy against cancer.

Adenovirus: Gene Vector

In addition to serving as an oncolytic virus, adenovirus can also function as a viral vector for cancer gene therapy. The adenoviral vectors are engineered such that viral genes are deleted and replaced with a cassette that expresses a foreign therapeutic gene. Typically the expression cassette is associated with a high-activity promoter such as the cytomegalovirus (CMV) immediate early promoter which efficiently drives expression of the foreign transgene. In addition to their capacity to serve as vaccines, adenovirus vectors have also received considerable attention for their role as gene delivery vehicles in cancer therapy. Studies by Shiba et al. examined the efficiency and selectivity of adenovirus gene delivery to HCC using hepatic artery administration followed by tumor vessel embolization [60]. The rationale for this strategy is that the secondary arterial embolization would allow the adenovirus particles to remain within the tumor vicinity to exert a more potent and selective gene transfer to tumor cells. These investigators went on to conclude through β-galactosidase staining that this approach of combining adenovirus vector (expressing β-gal) and degradable starch embolic microspheres did enable efficient gene transfer with a high tumor selectivity to HCC in rats [60]. Mutation of the tumor suppressor, p53, is one of the most common mutations in HCC biology and represents an attractive target for gene therapy. Restoration of wild-type p53 function in tumors through ectopic expression of exogenous p53 gene is anticipated

to suppress tumor growth through the induction of tumor cell death and cell cycle arrest. This was demonstrated by the work of Anderson et al. who showed that four daily hepatic artery treatments of recombinant adenovirus encoding wild-type p53 (rAd-p53) suppressed tumor growth when compared with untreated rats or animals treated with control adenovirus particles [61]. These findings demonstrate the potential for arterial gene delivery to tumors using recombinant adenoviruses, and support continued investigation of rAd-p53 gene therapy for liver malignancies. More recently this strategy was combined with TACE in a small cohort of patients with unresectable HCC [62]. This study found rAd-p53-based TACE could improve the overall survival (hazard ratio, 0.58; 95% confidence interval, 0.35–0.96; *P*, 0.035), progression-free survival (hazard ratio, 0.60; 95% confidence interval, 0.37–0.97; *P*, 0.037), and response rate (*P*, 0.047) compared with TACE monotherapy [62]. Patients receiving this combination therapy, however, did experience more occurrences of fever than with TACE alone ($P = 0.01$). Future larger-scale prospective randomized clinical trials are warranted to assess the efficacy and safety of rAd-p53-based TACE treatment for HCC.

Lipoprotein-Mediated Delivery of Omega-3 Fatty Acid

In recent years our own laboratory has developed a unique biologic nanomedicine for transarterial treatment of HCC. Our nanomedicine is engineered from circulating plasma low-density lipoproteins (LDL) (Fig. 9.9). These endogenous nanoscale lipid carriers are stripped of their neutral lipid cargo (cholesterol and triglycerides) and reconstituted with the natural polyunsaturated fatty acid, docosahexaenoic acid

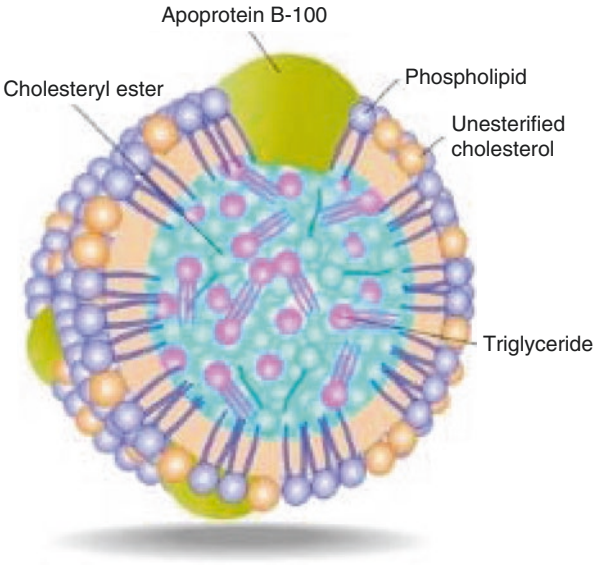

Fig. 9.9 Schematic diagram of plasma low-density lipoprotein (LDL). LDL a quasisperical particle that is approximately 20 nm in diameter. Overall the LDL particle is organized into two major domains, namely, a central apolar core of cholesteryl esters and triglycerides surrounded by an amphipathic shell consisting of a phospholipid monolayer, free unesterified cholesterol, and a single molecule of apolipoprotein B-100. (Credit: ellepigrafica/Shutterstock.com)

(DHA) [63]. We refer to this reconstituted LDL particle as LDL-DHA. Like its natural predecessor, LDL-DHA retains its affinity for LDL receptor and is readily taken up by peripheral tissues [63, 64]. Malignant tumors, in particular, are known to avidly sequester circulating LDL [65, 66]. Rapidly proliferating neoplastic tissues readily extract plasma LDL in order to acquire cholesterol to support membrane turnover and growth signaling [67, 68]. Tumor tissues also aggressively endocytose LDL-DHA nanoparticles expecting to acquire cholesterol; however the unintended deposit of DHA elicits cytotoxicity to the tumor cells. HCC like many other tumors maintains higher levels of oxidative stress compared to their normal counterparts [69–71]. The high levels of intracellular reactive oxygen species (ROS) can be highly destructive to cell membranes enriched with polyunsaturated fatty acids (PUFA). As such malignant tumors favor the expression of saturated and monounsaturated fatty acids in their lipid membranes over PUFAs [72]. Indeed, several independent studies have shown that HCC tissues have significantly lower polyunsaturation indices compared to adjacent nonmalignant liver [73, 74]. Thus in principle, LDL-DHA treatments reverse the lipid metabolic reprogramming of tumor cells by introducing PUFAs and sensitizing them to the damaging effects of their deviant redox biology [75]. The cellular demise of treated HCC is perpetuated as LDL-DHA selectively deregulates cellular antioxidant defenses (e.g., glutathione content, glutathione peroxidase 4 activity) and induces lethal lipid peroxidation in HCC cells in a dose-dependent manner [64, 76]. Transarterial delivery enables LDL-DHA nanoparticles to reach HCC tumors at high concentrations without systemic dilution. Unlike the traditional gelatin sponge or microsphere embolics which primarily lodge in peritumoral vessels [77, 78], the nanoscale size of LDL-DHA (22 nm) enables it to permeate into the tumor capillary bed and interact at the cell surface where it is endocytosed into tumor cells via receptor-mediated endocytosis. Preclinical studies revealed that following LDL-DHA treatment rat hepatomas (similar to in vitro cell experiments) selectively undergo marked oxidation of glutathione and NADPH couples, depletion of GPx4, and pronounced lipid peroxidation which culminate in extensive necrosis of the tumor (Fig. 9.10) [64]. This form of cell killing was recently described as ferroptosis [76], a non-apoptotic programed necrosis that is iron-dependent and characterized by the accumulation of lipid peroxides [79, 80]. Concurrently, the surrounding liver tissue is also exposed to LDL-DHA; however here it is well tolerated without redox disturbances or hepatic injury [64]. In normal hepatocytes DHA is likely enzymatically metabolized to nontoxic end products or esterified in membrane or neutral lipids. Studies are ongoing in our lab to elucidate the regulatory mechanisms governing the divergent metabolism of DHA in normal and malignant cells.

Multifaceted Antitumor Effects of DHA

To date, the anticancer actions of LDL-DHA are primarily attributed to the copious levels of lipid ROS generated in tumor cells. Recently numerous other groups have also investigated the effects of nanoparticle-free unesterified DHA and have

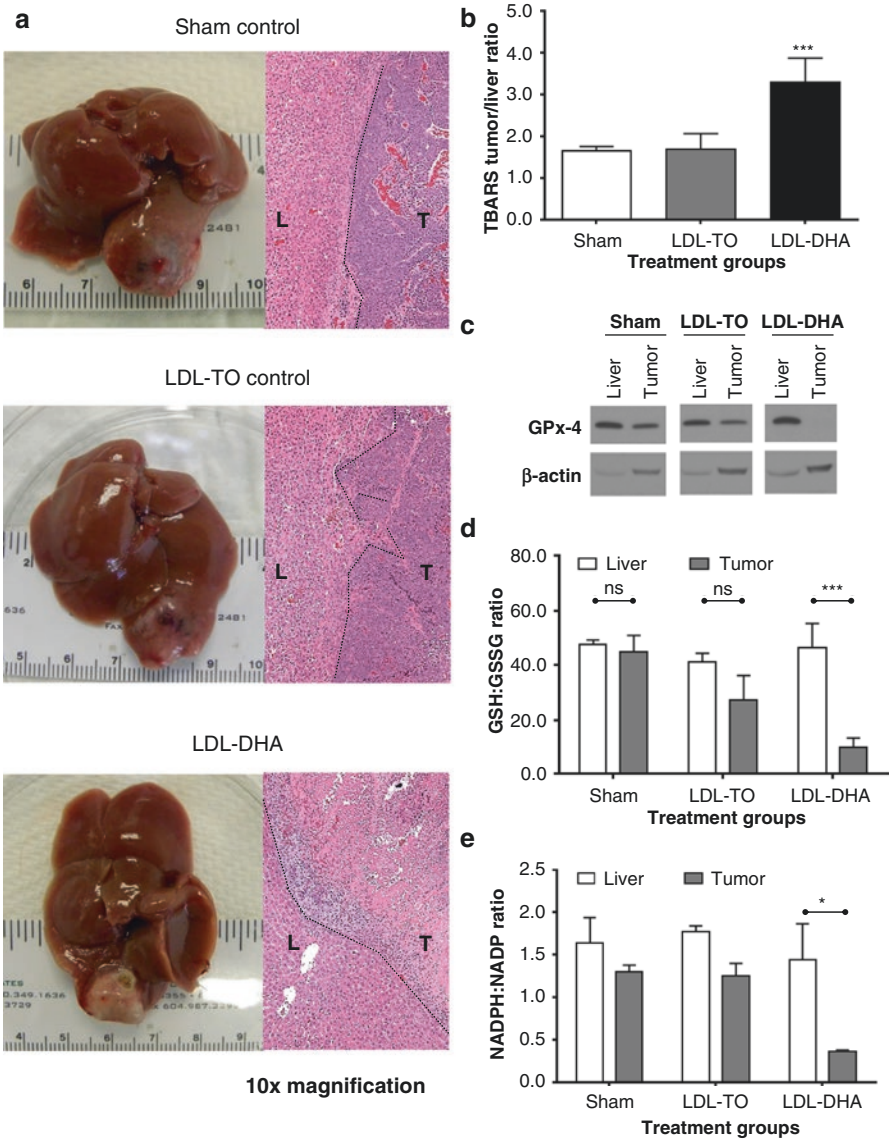

Fig. 9.10 Therapeutic effect of HAI of LDL-DHA in HCC-bearing rats. (**a**) Excised view of liver and hepatoma 3 days after sham or hepatic artery injection (HAI) with 2 mg/kg of LDL nanoparticles. The right panel shows the corresponding histology at the liver tumor interface (10× magnification). Dotted line indicates liver tumor boundary. (**b**) TBARS reading, (**c**) Glutathione peroxidase 4 (GPx4) protein expression. (**d**) GSH:GSSG and (**e**) NADPH:NADP levels in rat liver and hepatoma 72 h after Sham and HAI treatment of LDL nanoparticles (2 mg/kg).The data are expressed as a ratio of tumor to liver (mean ± SEM) for each treatment group. ∗, $P \leq 0.05$; ∗∗, $P \leq 0.01$; ∗∗∗, $P \leq 0.001$ versus indicated groups. (Adapted from Wen et al. 2016)

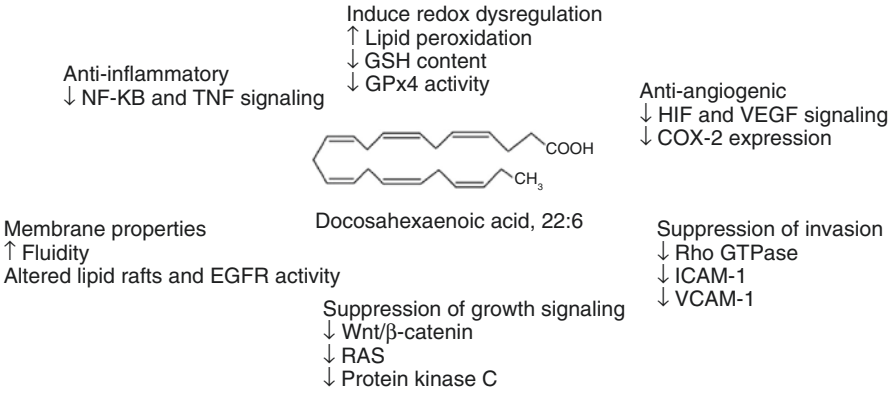

Fig. 9.11 Multifaceted anticancer effects of docosahexaenoic acid

implicated it in mediating multiple pathways of anticancer activity (see Fig. 9.11) [81–83]. Studies by Lim et al. have indicated that omega-3 PUFAs antagonize Wnt/β-catenin pathway signaling through modulation of GSK-3β/proteasome activity [84]. Thus by stabilizing the β-catenin degradation complex, DHA promotes β-catenin degradation. In this same study, the authors also reported that DHA inhibits prostaglandin E2 signaling by downregulating COX-2 expression while concurrently upregulating the COX-2 antagonist 15-hydroxyprostaglandin dehydrogenase [84]. These actions work in concert to suppress inflammation and tumor growth. Omega-3 PUFAs and their metabolites are also natural ligands for peroxisome proliferator-activated receptor (PPAR) [85, 86]. In this capacity DHA is able to suppress Iκκ/NF-κβ [87–89] and HIF-1α/VEGF signaling [90, 91]. The latter is particularly relevant, as conventional embolic therapies elicit a pronounced VEGF response which is associated with high rates of tumor recurrence [92, 93]. Other activities include the suppression of signaling growth factors (e.g., RAS and protein kinase C). DHA has also been cited to antagonize tumor spread and invasion through the downregulation of Rho GTPase, which inhibits cytoskeleton reorganization [94]. Finally, the incorporation of DHA into the cancer cell membrane can alter membrane properties by increasing fluidity, disrupting raft assembly and the signaling of membrane receptors such as EGFR [95]. Collectively, DHA is able to modulate multiple pathways of tumorigenesis which include cancer cell proliferation, differentiation, inflammation, angiogenesis, and metastasis. These anticancer attributes of DHA may also be active in the LDL nanoformulation. Thus, while LDL-DHA treatments primarily evoke tumor cytotoxicity through the generation of lipid radicals, secondary insults to the tumor may occur at a molecular level through the inhibition of inflammatory, angiogenic, and growth signaling pathways. In short, these properties potentially enable LDL-DHA to act as both a cytotoxic and a molecular targeted agent.

LDL-DHA Treatment Option for HCC

The components of the LDL-DHA nanomedicine, which include plasma LDL and DHA, are completely natural and biocompatible. Thus this technology readily lends itself for clinical translation. Furthermore, the selective tumor cytotoxicity of this agent makes it a safe and attractive candidate as a cancer therapeutic. In terms of treatment allocation, LDL-DHA could serve as an alternate therapy to patients who are contraindicated for TACE (i.e., impaired portal vein blood flow) or as a salvage therapy to patients who are unresponsive to TACE. Additionally, due to its selective antitumor activity, LDL-DHA therapy maybe indicated in patients with advanced HCC and poor liver function. In this setting the hepatic uptake of DHA would potentially provide anti-inflammatory and hepatoprotective benefits to the liver [96, 97] while at the same time provide tumor control. In summary, the LDL-DHA nanomedicine shows great promise as a novel complementary therapy to treat patients with HCC.

Conclusion and Future Outlook

The characteristic arterial hypervascularity of HCC, which is distinct from the predominant portal vascular supply to the surrounding liver parenchyma, provides a unique opportunity for therapeutic intervention. Several historic iterations of isolating, occluding, and finally embolizing the tumor vasculature have all served to preferentially starve the HCC of its blood supply to evoke tumor cell death (see Fig. 9.12). Refinements in interventional radiologic techniques, drug formulations, and patient selection have enabled transarterial therapies to emerge as the leading therapeutic option for patients with advanced HCC who cannot be effectively treated with surgical or ablative methods. TACE, either conventional or DEB-based, is recognized as the front-line therapy for treatment of HCC with BCLC-B disease. While both forms of TACE are considered therapeutically equivalent in providing similar survival benefits, lower incidences of some toxicities have been associated with DEB-TACE. More recently, TARE has emerged as an attractive alternate therapy due to its potential utility among patients with portal vein thrombus and added benefits of extended time to progression. Despite these advances TACE and TARE remain palliative therapies providing median overall survival of 20 months. Further refinements to HCC therapy should focus on increasing disease specificity beyond anatomic targeting to minimize collateral liver toxicity in patients with already compromised liver function. To this end, transarterial biomimetic drug delivery systems represent an unconventional and innovative approach to treat HCC. Biomimetic particulates leverage nature's optimized binding specificities to enable tumor-targeting therapeutic approaches. Unlike the indiscriminant actions of embolic or radiation-based agents, which often damage surrounding normal tissues, the biomimetic platforms specifically engage their tumor target at the cell surface to

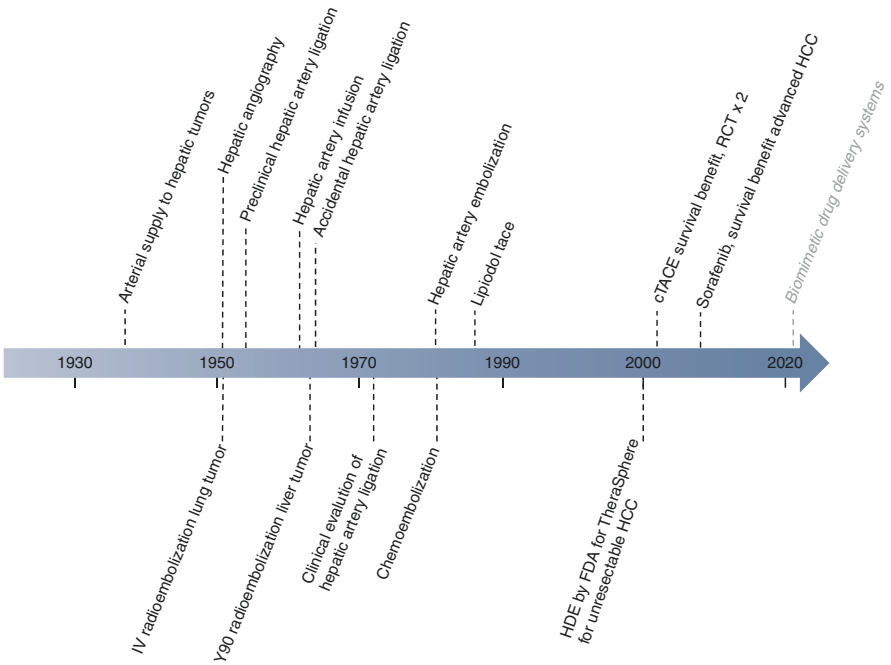

Fig. 9.12 Historic time line highlighting key advancements in transarterial treatment of hepatic tumors

subsequently elicit their intracellular cytotoxicities. The leukocyte, viral, and lipoprotein bioinspired constructs highlighted in this review represent emerging "out-of-the-box" strategies that offer the unique opportunity to interrogate tumors in a new and unprecedented manner to advance transarterial therapies beyond conventional treatment paradigms.

References

1. Wright RD. The blood supply of newly developed epithelial tissue in the liver. J Pathol Bacteriol. 1937;45(2):405–14.
2. Breedis C, Young G. The blood supply of neoplasms in the liver. Am J Pathol. 1954;30(5):969–77.
3. Markowitz J. The hepatic artery. Surg Gynecol Obstet. 1952;95(5):644–6.
4. Nilsson LA, Zettergren L. Blood supply and vascular pattern of induced primary hepatic carcinoma in rats. A microangiographic and histologic investigation. Acta Pathol Microbiol Scand. 1967;71(2):179–86.
5. Nilsson LA, Zettergren L. Effect of hepatic artery ligation on induced primary liver carcinoma in rats. Preliminary report. Acta Pathol Microbiol Scand. 1967;71(2):187–93.
6. Mori W, Masuda M, Miyanaga T. Hepatic artery ligation and tumor necrosis in the liver. Surgery. 1966;59(3):359–63.

7. Gelin LE, Lewis DH, Nilsson L. Liver blood flow in man during abdominal surgery. II. The effect of hepatic artery occlusion on the blood flow through metastatic tumor nodules. Acta Hepatosplenol. 1968;15(1):21–4.
8. Almersjo O, Bengmark S, Rudenstam CM, Hafstrom L, Nilsson LA. Evaluation of hepatic dearterialization in primary and secondary cancer of the liver. Am J Surg. 1972;124(1):5–9.
9. Bengmark S, Rosengren K. Angiographic study of the collateral circulation to the liver after ligation of the hepatic artery in man. Am J Surg. 1970;119(6):620–4.
10. Bierman HR, Byron RL Jr, Kelley KH, Grady A. Studies on the blood supply of tumors in man. III. Vascular patterns of the liver by hepatic arteriography in vivo. J Natl Cancer Inst. 1951;12(1):107–31.
11. Bierman HR, Kelly KH, Byron RL Jr, Dod KS, Shimkin MB. Studies on the blood supply of tumors in man. II. Intra-arterial nitrogen mustard therapy of cutaneous lesions. J Natl Cancer Inst. 1951;11(5):891–905.
12. Clarkson B, Young C, Dierick W, Kuehn P, Kim M, Berrett A, et al. Effects of continuous hepatic artery infusion of antimetabolites on primary and metastatic cancer of the liver. Cancer. 1962;15:472–88.
13. Chuang VP, Wallace S. Hepatic artery embolization in the treatment of hepatic neoplasms. Radiology. 1981;140(1):51–8. https://doi.org/10.1148/radiology.140.1.7244243.
14. Kato T, Nemoto R, Mori H, Takahashi M, Tamakawa Y, Harada M. Arterial chemoembolization with microencapsulated anticancer drug. An approach to selective cancer chemotherapy with sustained effects. JAMA. 1981;245(11):1123–7.
15. Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. Radiology. 1983;148(2):397–401. https://doi.org/10.1148/radiology.148.2.6306721.
16. Nakamura H, Hashimoto T, Oi H, Sawada S. Transcatheter oily chemoembolization of hepatocellular carcinoma. Radiology. 1989;170(3. Pt 1):783–6. https://doi.org/10.1148/radiology.170.3.2536946.
17. Takayasu K, Shima Y, Muramatsu Y, Moriyama N, Yamada T, Makuuchi M, et al. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. Radiology. 1987;163(2):345–51. https://doi.org/10.1148/radiology.163.2.3031724.
18. Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma--a randomized controlled trial. Gastroenterology. 1988;94(2):453–6.
19. Pelletier G, Roche A, Ink O, Anciaux ML, Derhy S, Rougier P, et al. A randomized trial of hepatic arterial chemoembolization in patients with unresectable hepatocellular carcinoma. J Hepatol. 1990;11(2):181–4.
20. Pelletier G, Ducreux M, Gay F, Luboinski M, Hagege H, Dao T, et al. Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization: a multicenter randomized trial. Groupe CHC. J Hepatol. 1998;29(1):129–34.
21. Bruix J, Llovet JM, Castells A, Montana X, Bru C, Ayuso MC, et al. Transarterial embolization versus symptomatic treatment in patients with advanced hepatocellular carcinoma: results of a randomized, controlled trial in a single institution. Hepatology. 1998;27(6):1578–83. https://doi.org/10.1002/hep.510270617.
22. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology. 2002;35(5):1164–71. https://doi.org/10.1053/jhep.2002.33156.
23. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet. 2002;359(9319):1734–9. https://doi.org/10.1016/S0140-6736(02)08649-X.
24. Brown KT, Do RK, Gonen M, Covey AM, Getrajdman GI, Sofocleous CT, et al. Randomized trial of hepatic artery embolization for hepatocellular carcinoma using doxorubicin-eluting microspheres compared with embolization with microspheres alone. J Clin Oncol. 2016;34(17):2046–53. https://doi.org/10.1200/JCO.2015.64.0821.

25. Lewis AL, Gonzalez MV, Lloyd AW, Hall B, Tang Y, Willis SL, et al. DC bead: in vitro characterization of a drug-delivery device for transarterial chemoembolization. J Vasc Interv Radiol. 2006;17(2. Pt 1):335–42. https://doi.org/10.1097/01.RVI.0000195323.46152.B3.
26. Lammer J, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, et al. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. Cardiovasc Intervent Radiol. 2010;33(1):41–52. https://doi.org/10.1007/s00270-009-9711-7.
27. Golfieri R, Giampalma E, Renzulli M, Cioni R, Bargellini I, Bartolozzi C, et al. Randomised controlled trial of doxorubicin-eluting beads vs conventional chemoembolisation for hepatocellular carcinoma. Br J Cancer. 2014;111(2):255–64. https://doi.org/10.1038/bjc.2014.199.
28. Bierman HR, Byron RL Jr, Miller ER, Shimkin MB. Effects of intra-arterial administration of nitrogen mustard. Am J Med. 1950;8(4):535.
29. Muller JH, Rossier PH. A new method for the treatment of cancer of the lungs by means of artificial radioactivity. Acta Radiol. 1951;35(5–6):449–68.
30. Grady ED, Sale WT, Rollins LC. Localization of radioactivity by intravascular injection of large radioactive particles. Ann Surg. 1963;157:97–114.
31. Caldarola L, Rosa U, Badellino F, Sosi S, Cavalli A. Preparation of 32-P labelled resin microspheres for radiation treatment of tumours by intra-arterial injection. Minerva Nucl. 1964;8:169–74.
32. Ariel IM. Treatment of inoperable primary pancreatic and liver cancer by the intra-arterial administration of radioactive isotopes (Y90 radiating microspheres). Ann Surg. 1965;162:267–78.
33. Blanchard RJ, Grotenhuis I, Lafave JW, Frye CW, Perry JF Jr. Treatment of experimental tumors; utilization of radioactive microspheres. Arch Surg. 1964;89:406–10.
34. Blanchard RJ, Lafave JW, Kim YS, Frye CS, Ritchie WP, Perry JF Jr. Treatment of patients with advanced cancer utilizing Y90 microspheres. Cancer. 1965;18:375–80.
35. Kim YS, Lafave JW, Maclean LD. The use of radiating microspheres in the treatment of experimental and human malignancy. Surgery. 1962;52:220–31.
36. Grady ED. Internal radiation therapy of hepatic cancer. Dis Colon Rectum. 1979;22(6):371–5.
37. Murthy R, Nunez R, Szklaruk J, Erwin W, Madoff DC, Gupta S, et al. Yttrium-90 microsphere therapy for hepatic malignancy: devices, indications, technical considerations, and potential complications. Radiographics. 2005;25(Suppl 1):S41–55. https://doi.org/10.1148/rg.25si055515.
38. Camacho JC, Moncayo V, Kokabi N, Reavey HE, Galt JR, Yamada K, et al. (90)Y Radioembolization: multimodality imaging pattern approach with angiographic correlation for optimized target therapy delivery. Radiographics. 2015;35(5):1602–18. https://doi.org/10.1148/rg.2015140314.
39. Salem R, Gabr A, Riaz A, Mora R, Ali R, Abecassis M, et al. Institutional decision to adopt Y90 as primary treatment for hepatocellular carcinoma informed by a 1,000-patient 15-year experience. Hepatology. 2017;68:1429–40. https://doi.org/10.1002/hep.29691.
40. Salem R, Gordon AC, Mouli S, Hickey R, Kallini J, Gabr A, et al. Y90 Radioembolization significantly prolongs time to progression compared with chemoembolization in patients with hepatocellular carcinoma. Gastroenterology. 2016;151(6):1155–63 e2. https://doi.org/10.1053/j.gastro.2016.08.029.
41. Padia SA, Johnson GE, Horton KJ, Ingraham CR, Kogut MJ, Kwan S, et al. Segmental yttrium-90 radioembolization versus segmental chemoembolization for localized hepatocellular carcinoma: results of a single-center, retrospective, propensity score-matched study. J Vasc Interv Radiol. 2017;28(6):777–85 e1. https://doi.org/10.1016/j.jvir.2017.02.018.
42. Les I, Doval E, Flavia M, Jacas C, Cardenas G, Esteban R, et al. Quality of life in cirrhosis is related to potentially treatable factors. Eur J Gastroenterol Hepatol. 2010;22(2):221–7. https://doi.org/10.1097/MEG.0b013e3283319975.
43. Marchesini G, Bianchi G, Amodio P, Salerno F, Merli M, Panella C, et al. Factors associated with poor health-related quality of life of patients with cirrhosis. Gastroenterology. 2001;120(1):170–8.

44. Orr JG, Homer T, Ternent L, Newton J, McNeil CJ, Hudson M, et al. Health related quality of life in people with advanced chronic liver disease. J Hepatol. 2014;61(5):1158–65. https://doi.org/10.1016/j.jhep.2014.06.034.
45. White JA, Gray SH, Li P, Simpson HN, McGuire BM, Eckhoff DE, et al. Current guidelines for chemoembolization for hepatocellular carcinoma: room for improvement? Hepatol Commun. 2017;1(4):338–46. https://doi.org/10.1002/hep4.1046.
46. Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. Am J Transplant. 2010;10(4 Pt 2):1003–19. https://doi.org/10.1111/j.1600-6143.2010.03037.x.
47. Parodi A, Molinaro R, Sushnitha M, Evangelopoulos M, Martinez JO, Arrighetti N, et al. Bio-inspired engineering of cell- and virus-like nanoparticles for drug delivery. Biomaterials. 2017;147:155–68. https://doi.org/10.1016/j.biomaterials.2017.09.020.
48. Yoo JW, Irvine DJ, Discher DE, Mitragotri S. Bio-inspired, bioengineered and biomimetic drug delivery carriers. Nat Rev Drug Discov. 2011;10(7):521–35. https://doi.org/10.1038/nrd3499.
49. Dehaini D, Fang RH, Zhang L. Biomimetic strategies for targeted nanoparticle delivery. Bioeng Transl Med. 2016;1(1):30–46. https://doi.org/10.1002/btm2.10004.
50. Chan CJ, Smyth MJ, Martinet L. Molecular mechanisms of natural killer cell activation in response to cellular stress. Cell Death Differ. 2013;21:5. https://doi.org/10.1038/cdd.2013.26.
51. Vivier E, Ugolini S. Natural killer cells: from basic research to treatments. Front Immunol. 2011;2:18. https://doi.org/10.3389/fimmu.2011.00018.
52. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013):44–9. https://doi.org/10.1126/science.1198687.
53. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, Yu SJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology. 2015;148(7):1383–91 e6. https://doi.org/10.1053/j.gastro.2015.02.055.
54. Yu R, Yang B, Chi X, Cai L, Liu C, Yang L, et al. Efficacy of cytokine-induced killer cell infusion as an adjuvant immunotherapy for hepatocellular carcinoma: a systematic review and meta-analysis. Drug Des Devel Ther. 2017;11:851–64. https://doi.org/10.2147/DDDT.S124399.
55. Sheu AY, Zhang Z, Omary RA, Larson AC. MRI-monitored transcatheter intraarterial delivery of SPIO-labeled natural killer cells to hepatocellular carcinoma: preclinical studies in a rodent model. Investig Radiol. 2013;48(6):492–9. https://doi.org/10.1097/RLI.0b013e31827994e5.
56. Su Z, Wang X, Zheng L, Lyu T, Figini M, Wang B, et al. MRI-guided interventional natural killer cell delivery for liver tumor treatment. Cancer Med. 2018;7(5):1860–9. https://doi.org/10.1002/cam4.1459.
57. Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med. 2000;6(7):821–5. https://doi.org/10.1038/77558.
58. Ebert O, Shinozaki K, Huang TG, Savontaus MJ, Garcia-Sastre A, Woo SL. Oncolytic vesicular stomatitis virus for treatment of orthotopic hepatocellular carcinoma in immune-competent rats. Cancer Res. 2003;63(13):3605–11.
59. Shinozaki K, Ebert O, Suriawinata A, Thung SN, Woo SL. Prophylactic alpha interferon treatment increases the therapeutic index of oncolytic vesicular stomatitis virus virotherapy for advanced hepatocellular carcinoma in immune-competent rats. J Virol. 2005;79(21):13705–13. https://doi.org/10.1128/JVI.79.21.13705-13713.2005.
60. Shiba H, Misawa T, Iida T, Okamoto T, Futagawa Y, Sakurai M, et al. Adenovirus vector-mediated gene therapy using iodized oil esters for hepatocellular carcinoma in rats. Anticancer Res. 2008;28(1a):51–3.
61. Anderson SC, Johnson DE, Harris MP, Engler H, Hancock W, Huang WM, et al. p53 gene therapy in a rat model of hepatocellular carcinoma: intra-arterial delivery of a recombinant adenovirus. Clin Cancer Res. 1998;4(7):1649–59.
62. Shen A, Liu S, Yu W, Deng H, Li Q. p53 gene therapy-based transarterial chemoembolization for unresectable hepatocellular carcinoma: a prospective cohort study. J Gastroenterol Hepatol. 2015;30(11):1651–6. https://doi.org/10.1111/jgh.13009.

63. Reynolds L, Mulik RS, Wen X, Dilip A, Corbin IR. Low-density lipoprotein-mediated delivery of docosahexaenoic acid selectively kills murine liver cancer cells. Nanomedicine (Lond). 2014;9(14):2123–41. https://doi.org/10.2217/nnm.13.187.
64. Wen X, Reynolds L, Mulik RS, Kim SY, Van Treuren T, Nguyen LH, et al. Hepatic arterial infusion of low-density lipoprotein docosahexaenoic acid nanoparticles selectively disrupts redox balance in hepatoma cells and reduces growth of orthotopic liver tumors in rats. Gastroenterology. 2016;150(2):488–98. https://doi.org/10.1053/j.gastro.2015.10.008.
65. Vitols S. Uptake of low-density lipoprotein by malignant cells--possible therapeutic applications. Cancer Cells. 1991;3(12):488–95.
66. Firestone RA. Low-density lipoprotein as a vehicle for targeting antitumor compounds to cancer cells. Bioconjug Chem. 1994;5(2):105–13.
67. Guo D, Reinitz F, Youssef M, Hong C, Nathanson D, Akhavan D, et al. An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/SREBP-1/LDLR–dependent pathway. Cancer Discov. 2011;1:442–56. https://doi.org/10.1158/2159-8290.cd-11-0102.
68. Rodrigues dos Santos C, Domingues G, Matias I, Matos J, Fonseca I, de Almeida JM, et al. LDL-cholesterol signaling induces breast cancer proliferation and invasion. Lipids Health Dis. 2014;13:16. https://doi.org/10.1186/1476-511X-13-16.
69. Czeczot H, Scibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta Biochim Pol. 2006;53(1):237–42.
70. Tsai S-M, Lin S-K, Lee K-T, Hsiao J-K, Huang J-C, Wu S-H, et al. Evaluation of redox statuses in patients with hepatitis B virus-associated hepatocellular carcinoma. Ann Clin Biochem. 2009;46(5):394–400. https://doi.org/10.1258/acb.2009.009029.
71. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett. 1995;358(1):1–3. https://doi.org/10.1016/0014-5793(94)01368-B.
72. Rysman E, Brusselmans K, Scheys K, Timmermans L, Derua R, Munck S, et al. De novo lipogenesis protects cancer cells from free radicals and chemotherapeutics by promoting membrane lipid saturation. Cancer Res. 2010;70(20):8117–26. https://doi.org/10.1158/0008-5472.CAN-09-3871.
73. Skill NJ, Scott RE, Wu J, Maluccio MA. Hepatocellular carcinoma associated lipid metabolism reprogramming. J Surg Res. 2011;169(1):51–6. https://doi.org/10.1016/j.jss.2009.09.005.
74. Araki E, Phillips F, Privett OS. Studies on lipid and fatty acid composition of human hepatoma tissue. Lipids. 1974;9(9):707–12.
75. Moss LR, Mulik RS, Van Treuren T, Kim SY, Corbin IR. Investigation into the distinct subcellular effects of docosahexaenoic acid loaded low-density lipoprotein nanoparticles in normal and malignant murine liver cells. Biochim Biophys Acta. 2016;1860:2363–76. https://doi.org/10.1016/j.bbagen.2016.07.004.
76. Ou W, Mulik RS, Anwar A, McDonald JG, He X, Corbin IR. Low-density lipoprotein docosahexaenoic acid nanoparticles induce ferroptotic cell death in hepatocellular carcinoma. Free Radic Biol Med. 2017;112:597–607. https://doi.org/10.1016/j.freeradbiomed.2017.09.002.
77. Wáng Y-XJ, De Baere T, Idée J-M, Ballet S. Transcatheter embolization therapy in liver cancer: an update of clinical evidences. Chin J Cancer Res. 2015;27(2):96–121. https://doi.org/10.3978/j.issn.1000-9604.2015.03.03.
78. Namur J, Citron SJ, Sellers MT, Dupuis MH, Wassef M, Manfait M, et al. Embolization of hepatocellular carcinoma with drug-eluting beads: doxorubicin tissue concentration and distribution in patient liver explants. J Hepatol. 2011;55(6):1332–8. https://doi.org/10.1016/j.jhep.2011.03.024.
79. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, et al. Regulation of ferroptotic cancer cell death by GPX4. Cell. 2014;156(1–2):317–31. https://doi.org/10.1016/j.cell.2013.12.010.
80. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060–72. https://doi.org/10.1016/j.cell.2012.03.042.
81. Manzi L, Costantini L, Molinari R, Merendino N. Effect of dietary ω-3 polyunsaturated fatty acid DHA on glycolytic enzymes and Warburg phenotypes in cancer. Biomed Res Int. 2015;2015:137097. https://doi.org/10.1155/2015/137097.

82. Stephenson JA, Al-Taan O, Arshad A, Morgan B, Metcalfe MS, Dennison AR. The multifaceted effects of omega-3 polyunsaturated fatty acids on the hallmarks of cancer. J Lipids. 2013;2013:261247. https://doi.org/10.1155/2013/261247.
83. D'Eliseo D, Velotti F. Omega-3 fatty acids and cancer cell cytotoxicity: implications for multitargeted cancer therapy. J Clin Med. 2016;5(2):15. https://doi.org/10.3390/jcm5020015.
84. Lim K, Han C, Dai Y, Shen M, Wu T. Omega-3 polyunsaturated fatty acids inhibit hepatocellular carcinoma cell growth through blocking beta-catenin and cyclooxygenase-2. Mol Cancer Ther. 2009;8(11):3046–55. https://doi.org/10.1158/1535-7163.MCT-09-0551.
85. Clarke SD, Thuillier P, Baillie RA, Sha X. Peroxisome proliferator-activated receptors: a family of lipid-activated transcription factors. Am J Clin Nutr. 1999;70(4):566–71. https://doi.org/10.1093/ajcn/70.4.566.
86. Gani OA. Are fish oil omega-3 long-chain fatty acids and their derivatives peroxisome proliferator-activated receptor agonists? Cardiovasc Diabetol. 2008;7:6. https://doi.org/10.1186/1475-2840-7-6.
87. He X, Liu W, Shi M, Yang Z, Zhang X, Gong P. Docosahexaenoic acid attenuates LPS-stimulated inflammatory response by regulating the PPARγ/NF-κB pathways in primary bovine mammary epithelial cells. Res Vet Sci. 2017;112:7–12. https://doi.org/10.1016/j.rvsc.2016.12.011.
88. Liao Z, Dong J, Wu W, Yang T, Wang T, Guo L, et al. Resolvin D1 attenuates inflammation in lipopolysaccharide-induced acute lung injury through a process involving the PPARgamma/NF-kappaB pathway. Respir Res. 2012;13:110. https://doi.org/10.1186/1465-9921-13-110.
89. Yang YC, Lii CK, Wei YL, Li CC, Lu CY, Liu KL, et al. Docosahexaenoic acid inhibition of inflammation is partially via cross-talk between Nrf2/heme oxygenase 1 and IKK/NF-kappaB pathways. J Nutr Biochem. 2013;24(1):204–12. https://doi.org/10.1016/j.jnutbio.2012.05.003.
90. Mouradian M, Kikawa KD, Dranka BP, Komas SM, Kalyanaraman B, Pardini RS. Docosahexaenoic acid attenuates breast cancer cell metabolism and the Warburg phenotype by targeting bioenergetic function. Mol Carcinog. 2015;54(9):810–20. https://doi.org/10.1002/mc.22151.
91. Zhou J, Zhang S, Xue J, Avery J, Wu J, Lind SE, et al. Activation of peroxisome proliferator-activated receptor alpha (PPARalpha) suppresses hypoxia-inducible factor-1alpha (HIF-1alpha) signaling in cancer cells. J Biol Chem. 2012;287(42):35161–9. https://doi.org/10.1074/jbc.M112.367367.
92. Xiong ZP, Yang SR, Liang ZY, Xiao EH, Yu XP, Zhou SK, et al. Association between vascular endothelial growth factor and metastasis after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2004;3(3):386–90.
93. Liu K, Min X-L, Peng J, Yang K, Yang L, Zhang X-M. The changes of HIF-1α and VEGF expression after TACE in patients with hepatocellular carcinoma. J Clin Med Res. 2016;8(4):297–302. https://doi.org/10.14740/jocmr2496w.
94. Yi L, Zhang QY, Mi MT. [Role of Rho GTPase in inhibiting metastatic ability of human prostate cancer cell line PC-3 by omega-3 polyunsaturated fatty acid]. Ai Zheng. 2007;26(12):1281–6.
95. Schley PD, Brindley DN, Field CJ. (n-3) PUFA alter raft lipid composition and decrease epidermal growth factor receptor levels in lipid rafts of human breast cancer cells. J Nutr. 2007;137(3):548–53. https://doi.org/10.1093/jn/137.3.548.
96. Gonzalez-Periz A, Planaguma A, Gronert K, Miquel R, Lopez-Parra M, Titos E, et al. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. FASEB J. 2006;20(14):2537–9. https://doi.org/10.1096/fj.06-6250fje.
97. Depner CM, Philbrick KA, Jump DB. Docosahexaenoic acid attenuates hepatic inflammation, oxidative stress, and fibrosis without decreasing hepatosteatosis in a Ldlr(−/−) mouse model of western diet-induced nonalcoholic steatohepatitis. J Nutr. 2013;143(3):315–23. https://doi.org/10.3945/jn.112.171322.

Chapter 10
Precision Locoregional Therapies for Hepatocellular Carcinoma: Percutaneous Ablation and Radiotherapy

Ryosuke Tateishi and Naoto Fujiwara

Introduction

Hepatocellular carcinoma (HCC) generally arises from chronically diseased liver with impaired function, which often limits application of surgical therapies (see Chap. 8). In addition, even if regular HCC screening program is widely adopted as in Japan, only one-third of HCC patients are diagnosed at early stage and eligible for surgical resection. Thus, locoregional therapies have been developed to expand treatment options for locally limited but more advanced HCC tumors outside indication of surgical therapies and to improve outcome of surgical therapies. In this chapter, we overview the two major locoregional treatment approaches, percutaneous ablation and radiotherapy, with special focus on recent technical development to improve precision of the treatment and maximize therapeutic benefit (see Chap. 9 for interventional radiologic therapies).

R. Tateishi (✉)
Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
e-mail: tateishi-tky@umin.ac.jp

N. Fujiwara

Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_10

Percutaneous Ablation

Percutaneous ablation is a method to destroy targeted tumor by chemical reaction, heat, freezing, or electric pulse using needle-like devices. Percutaneous ethanol injection is the first ablative method applied for HCC by Japanese hepatologists in the early 1980s. Since then, together with its successor techniques, percutaneous ablation has been widely used as a modality to achieve high local tumor control and incorporated in HCC treatment algorithms globally [1–3].

Indication

The original indication for ethanol injection was somewhat arbitrarily defined as three or fewer tumors, none of which exceed 3 cm in diameter (3/3 rule) [4], which has been adopted in most practice guidelines until now [1–3]. Percutaneous ablation can achieve local tumor control rate comparable to surgical resection and better than other locoregional therapies such as transarterial chemoembolization (TACE) in small HCC tumors, whereas the control rate decrease as tumor diameter increases [5]. TACE will have advantage in treating multifocal lesions more than three. Interestingly, the empirical 3/3 rule distinguishes more aggressive tumors reasonably well and has also been utilized as a part of indication criteria for liver transplantation [6]. Recent technical advancement has enabled to achieve larger ablation zone, which may lead to expansion of indication criteria. Percutaneous ablation can also be applicable to inoperable cases due to decompensated cirrhosis if liver function is preserved at Child-Pugh class A or B, although survival benefit for Child-Pugh class C patients is minimal [7].

Ablative Methods

Radiofrequency Ablation

Radiofrequency ablation (RFA) is a thermal ablation method, using heat produced by electric current. RFA utilizes alternating current of 450 kHz, which is transmitted from the inserted electrode tip through patients' body to the grounding pad pasted on the back or the thigh and induces heat coagulation of targeted tumor [8, 9]. RFA was introduced as a treatment modality for HCC, following ethanol injection and the first-generation microwave ablation. RFA overcome the limitation of ethanol injection, which is effective only for small tumors (e.g., <2 cm in diameter) with capsule and without intra-tumoral septa, by producing larger ablation zone independent of capsule and septa [10]. Multiple studies have demonstrated superior local tumor control and posttreatment survival for RFA compared to ethanol injection [11, 12]. RFA is currently the most widely used percutaneous ablation method.

There are two types of RFA electrode, single-needle and expandable electrodes (Fig. 10.1) [13]. An internal cooling system is equipped inside the single-needle electrode to prevent reducing ablation zone due to burnt tissue sticking on the surface of the needle. The expandable electrode equips four to nine small electrodes in the inner sheath that can be expanded near the target tumor to produce a wide ablation zone. The advantage of the expandable electrode over single-needle electrode is secured ablation zone inside the expanded small electrodes, which can reach 5 cm in diameter. Disadvantage of the expandable electrode is potential injury of the vasculature by the tip of small electrodes, which could also cause draining of electric power through the punctured vessels and incomplete ablation. In addition, its thicker outer sheath (15 gauge [G]) compared to the single-needle electrode (17 G) is another limitation that may increase the risk of bleeding and tumor cell seeding. To date, the second-generation microwave ablation can produce similar or even larger ablation zone to the expandable electrode, and as a consequence, the expandable electrode has been less frequently used. The multi-electrode system is a variation of the single-needle electrode, where up to three single-needle electrodes are inserted in parallel to achieve larger ablation zone or

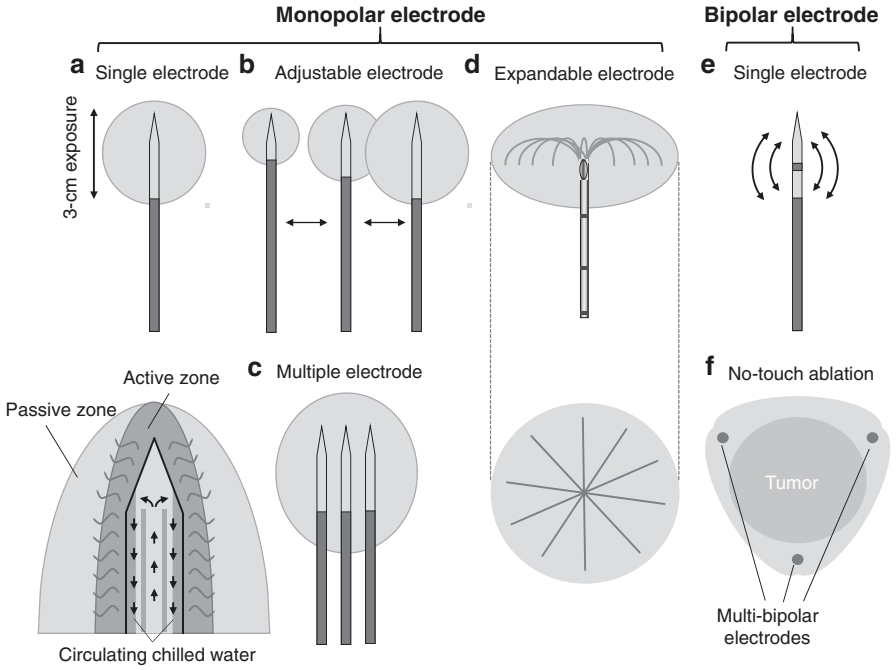

Fig. 10.1 Various devices for radiofrequency ablation. (**a**) Single electrode with internal cooling. (**b**) Adjustable electrode with variable ablative zone with one electrode. (**c**) Multiple monopolar electrodes with switch controller to enable larger ablative zone. (**d**) Expandable electrode with four to nine smaller electrodes inside. (**e**) Bipolar electrode with no need of grounding pad. (**f**) Multi-bipolar system for no-touch ablation by placing the electrodes to surround but not directly puncture the target tumor

to avoid direct puncture of targeted lesion [14]. Either of monopolar or bipolar electrodes are used in the system, and the latter is free from using grounding pads and associated complications [15].

Microwave Ablation

Microwave ablation (MWA) has been developed since the early 1990s [16]. An electrode connected to a microwave generator is inserted through a 14 G guide needle under ultrasonographic guidance. Microwaves of 2450 MHz are emitted from the tip of the electrode that can create an up to 1.5-cm-width ablation zone (Fig. 10.2). The first-generation microwave had a clear advantage over ethanol injection with the secured ablation zone, not affected by intra-tumoral septa that blocks ethanol penetration. On the other hand, the thick needle is a disadvantage that increases the risk of complications such as intraperitoneal hemorrhage and neoplastic seeding. The first-generation MWA was soon taken over by the single-needle RFA, which can achieve similar ablation zone with thinner needle (17 G) in the 2000s [17]. Recently developed second-generation MWA yields larger ablation zone than the single-needle RFA in a shorter

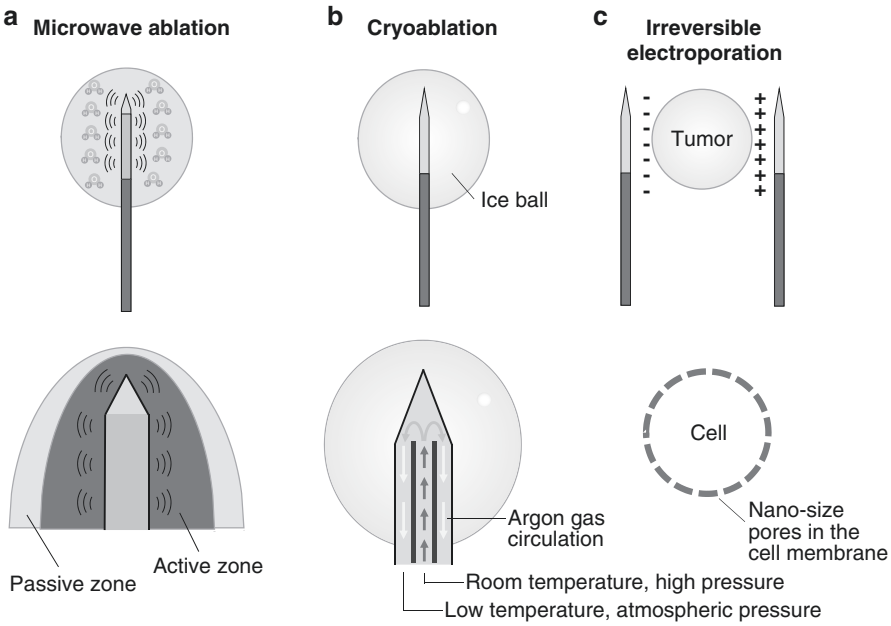

Fig. 10.2 Various ablation technologies. (**a**) Microwave ablation. Microwaves of 2450 MHz are emitted from the tip of the electrode, which directly heat water in the surrounding tissue. Microwaves can propagate over surrounding vessels in the active zone. Passive zone is heated by conduction. (**b**) Cryoablation. Tissue freezing is induced by Joule-Thomson effect using argon or helium gas. (**c**) Irreversible electroporation. Very high-intensity electric pulses between two electrodes make irreversible nano-size pores in the cell membrane

ablation time. In addition, compared to RFA, MWA is less affected by "heat-sink effect," which restricts ablation zone due to blood flow adjacent to the electrode, because microwave can propagate over surrounding vessels within the active zone [18]. These advantages of the second-generation MWA have helped revive it as a viable option of locoregional HCC treatment. Recent reports suggest that the second-generation MWA is superior to single-needle RFA in terms of local tumor control [19, 20].

Ethanol Injection

Percutaneous ethanol injection is the precursor of all percutaneous ablation techniques, which was first described in 1983 [21]. In the original procedure, a 22 G hollow needle with stylet is inserted into the tumor under sonographic guidance, and then 2–8 mL of absolute ethanol is injected through the needle and infiltrates into the tumor via sinusoid-like structure and causes coagulation necrosis [4]. Ethanol injection is no longer performed at tertiary centers, where a high volume of HCC patients are treated. However, ethanol injection is an inexpensive procedure using readily available materials and with proven antitumor effect for small HCC tumors. Therefore, it still has a role in daily clinical practice when RFA and MWA are not accessible [22].

Cryoablation

In contrast to the heat or chemical ablation techniques, cryoablation uses low temperatures (< -20 °C) to induce tissue necrosis [23]. Tissue freezing is induced by Joule-Thomson effect via a probe using argon or helium gas. An ice ball around the probe is indicative of irreversible cellular damage with intracellular ice crystals. Small vascular vessels are frozen and occluded, whereas large vessels are maintained. The first-generation cryoablation required a thick (2.2 mm, approximately 11 G) probe and often caused bleeding complications and cryoshock, systemic inflammatory syndrome with multi-organ failure following large tissue freezing [24]. Recent technical development such as the thinner (17 G) probe and multi-probe system may advance cryoablation as an alternative strategy to RFA [25, 26].

Irreversible Electroporation

Irreversible electroporation (IRE) is a nonthermal ablation, using very high-intensity electric pulse between two electrodes that causes irreversible pore formation in cell membrane lipid bilayer and results in cell death [27]. Unlike other ablative methods, the connective tissue, basal membranes, and lumens of the vasculature are relatively preserved in IRE [28]. This feature will mitigate technical barrier to ablate tumor adjacent to bile ducts and hepatic hilum with RFA and MWA. On the other hand, general anesthesia is required with a muscular blockade to prevent muscle spasm. Cardiac arrhythmia and use of pacemakers are contraindications.

Techniques for Precision Percutaneous Ablation

The major limitations of percutaneous ablation include restricted applicability to difficult-to-access regions, such as hepatic hilum and dome, and visibility of target tumor under ultrasound to guide needle/probe insertion. Several techniques have been developed to address the challenges, most of which can be readily applicable in daily clinical care of the patients.

Artificial Pleural Effusion

Ultrasonographic visualization of HCC tumor located in hepatic dome right beneath the diaphragm is often challenging. Some tumors can be visualized by adjusting patient posture (e.g., head-up or sitting position) or tilting the operation table. Laparoscopic or CT-guided techniques can be employed, although these approaches are highly resource-intensive. Artificial pleural effusion is a simple alternative method to address the issue, which can be conveniently and inexpensively utilized in daily clinical practice. Five percent glucose solution, which is spontaneously absorbed, is infused into the pleural cavity via a 14 G and metallic needle with a stylet to create an acoustic window (Fig. 10.3) [29]. This procedure can be safely performed with low risk of respiratory insufficiency [30]. Artificial pleural effusion is contraindicated in patients who had left pneumonectomy and may not improve tumor visualization when pleural adhesion is present.

Artificial Ascites

Artificial ascites is a similar fluid infusion-based method, injecting the fluid into the peritoneal cavity, to mainly improve therapeutic access to index tumor adjacent to other organs, especially the intestine, to avoid perforation (Figs. 10.4,

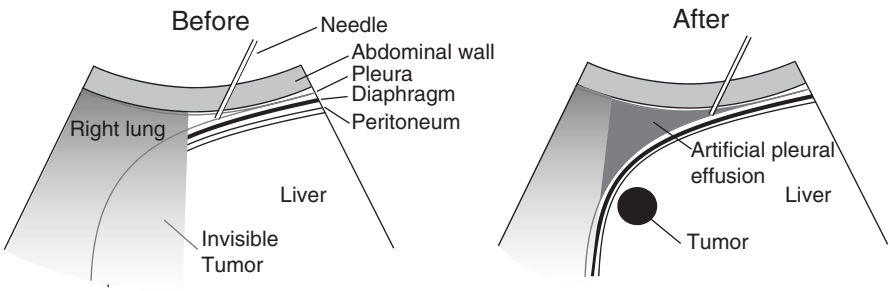

Fig. 10.3 Artificial pleural effusion technique. A 500 mL of 5% glucose solution is infused into the pleural cavity to achieve clear visualization of the tumor located beneath the diaphragm

and 10.5). Artificial ascites can also be used to improve visualization of tumors in the hepatic dome like artificial pleural effusion, although larger amount of fluid needs to be infused [31, 32]. Artificial ascites may not decrease the risk of intestinal perforation or penetration in a case there is adhesion between the liver and surrounding intestine.

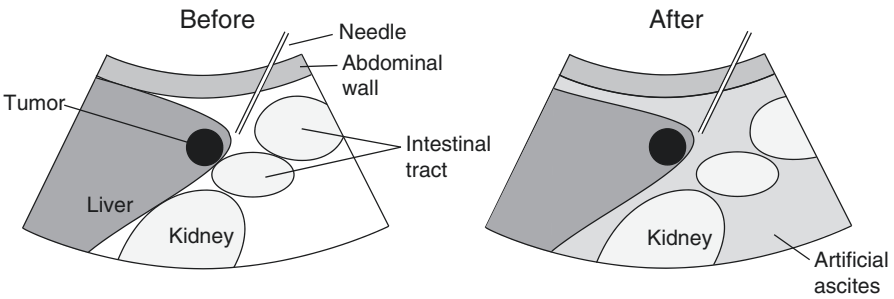

Fig. 10.4 Artificial ascites technique. A 5% glucose solution is infused to create a space between the liver and adjacent organs to enable safe ablation

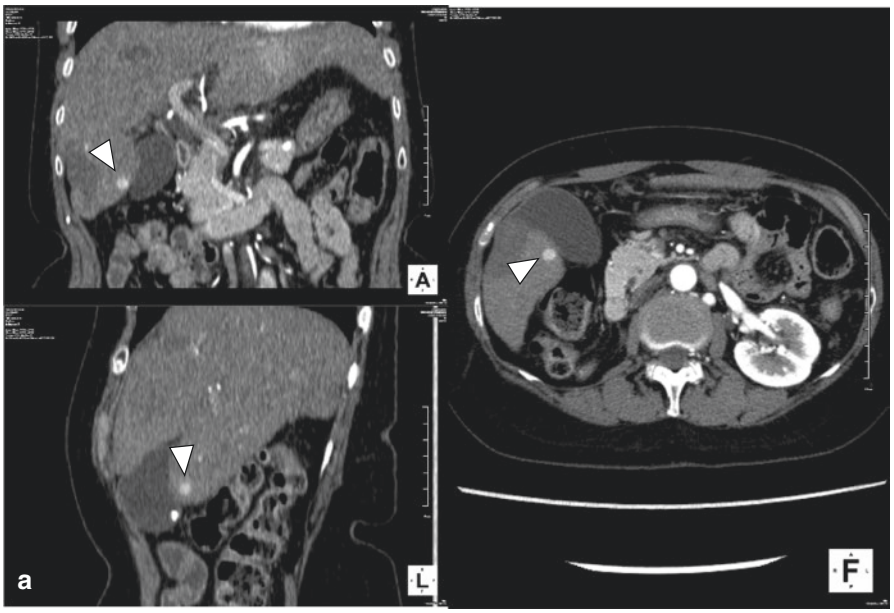

Fig. 10.5 Representative case with recurrent HCC treated with artificial ascites. (**a**) Enhanced CT before ablation. A small nodule in segment 6 adjacent to the gallbladder and the ascending colon (allow head). (**b**) An ultrasonographic image during ablation. An electrode was inserted to the tumor after the infusion of 5% glucose solution. (**c**) Enhanced CT after ablation. The target was completely ablated without any damage to adjacent organs (arrow head)

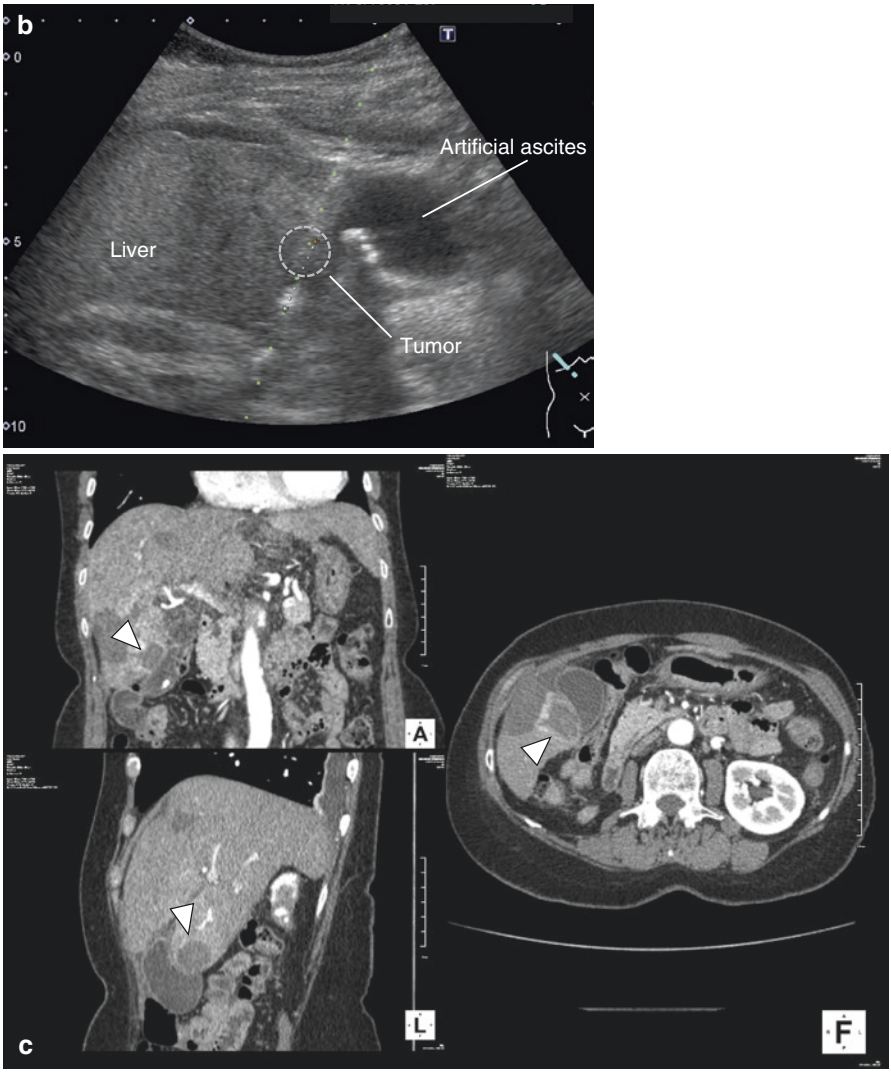

Fig. 10.5 (continued)

Contrast Ultrasonography

Besides tumor location, various factors such as coarse liver parenchyma, small tumor size, and scar created by previous treatment impede clear visualization of target tumor. Contrast agents for ultrasonography have been developed to obtain vascular images on B-mode or Doppler ultrasonography. Levovist, the first-generation contrast agent that contains a suspension of galactose microparticles, has improved differential diagnosis of liver tumors. However, Levovist is not suitable for percutaneous ablation because of short-lasting enhancement. Sonazoid, a second-generation sonographic contrast agent, contains a lipid-stabilized suspension of perfluorobutane gas microbubbles and yields long-lasting enhancement. In addition, the perfluorobutane

microbubbles are taken up by Kupffer cells and enable parenchymal enhancement approximately 15 min after the injection (Kupffer phase), in which malignant liver tumors are visualized as defect in enhanced non-cancerous liver parenchyma (Fig. 10.6). The Sonazoid Kupffer phase image can be conveniently used to assist percutaneous ablation procedure since the phase lasts more than 30 min and is easily reproduced by another injection of the agent [33, 34].

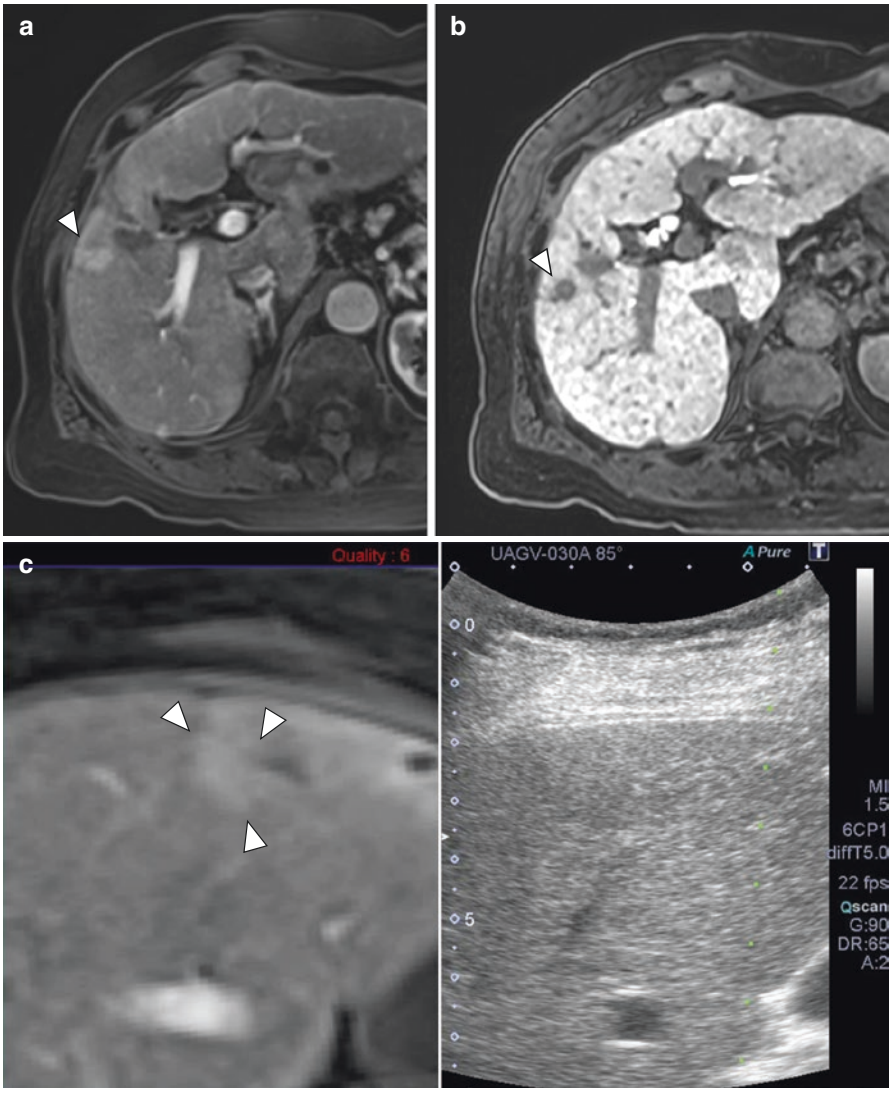

Fig. 10.6 (**a**, **b**) A case with HCC treated with radiofrequency ablation using fusion imaging and contrast ultrasonography. (**c**) A fusion imaging before contrast ultrasonography. The left panel shows a virtual image of the tumor constructed from previously taken in advance. The tumor cannot be clearly visualized on ultrasonography (right panel) before contrast media injection. (**d**) A fusion imaging after Sonazoid injection. The target tumor with small satellite nodule (arrow head) was visualized on Kupffer phase 15 min after the injection of contrast media

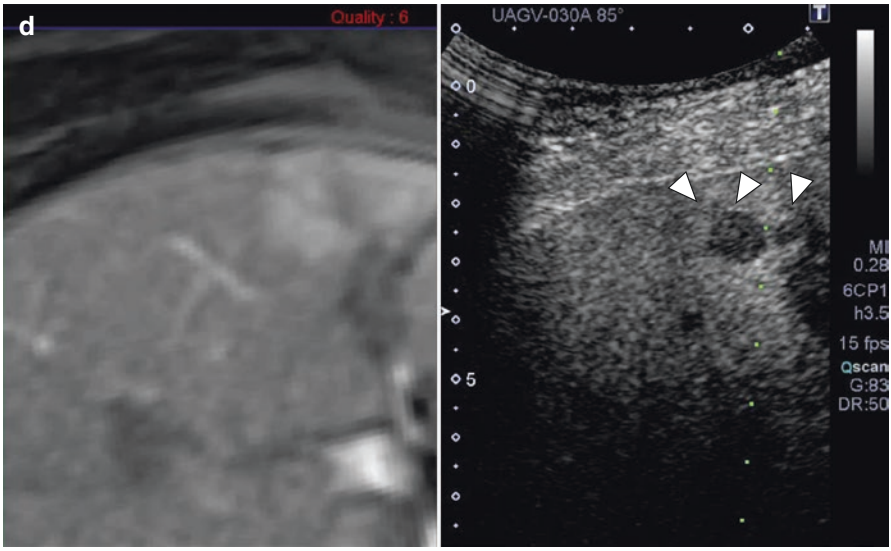

Fig. 10.6 (continued)

Fusion Imaging

Fusion imaging is a real-time construction of virtual sonographic image on the monitor of ultrasonographic equipment using CT or MRI images obtained in advance (Figs. 10.6 and 10.7) [35]. The system consists of an ultrasonographic apparatus, a magnetic field generator, and a magnetic sensor attached to the ultrasound probe. First, the digital data of CT or MRI images are uploaded to the ultrasonographic apparatus. After registration of specific points on the real ultrasonographic image to the virtual image, real-time virtual image synchronized with the movement of the ultrasound probe can be obtained. Fusion imaging significantly decreases the risk of mistargeting and sometimes enable accurate ablation of invisible tumor nodules on ultrasonography. The system can be combined with contrast ultrasonography, which enables more accurate and safe ablation in difficult-to-treat cases [36].

Short- and Long-term Outcomes

Local Tumor Progression

Local tumor progression is a key short-term treatment efficacy measure, which is typically defined as appearance of newly diagnosed tumor adjacent to previously ablated site. It has been demonstrated that local tumor progression rate after RFA is generally lower compared to ethanol injection [11, 12]. However, the reported local tumor progression rates at 5 years in RFA vary from 3.2% to 27% even in high-volume centers [7, 37]. This is likely due to variation in pretreatment detection of small satellite

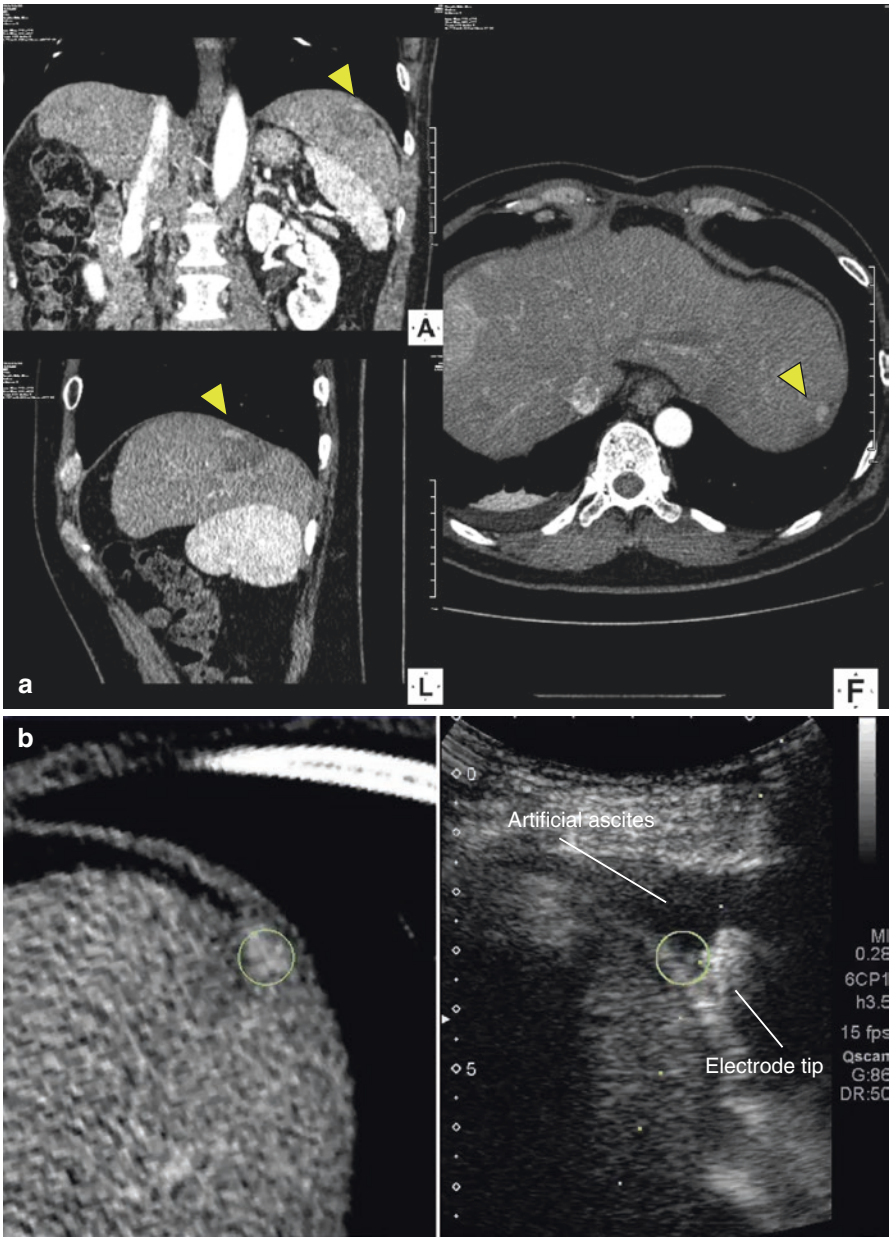

Fig. 10.7 Representative case treated with radiofrequency ablation using the fusion imaging and artificial pleural effusion. (**a**) A small HCC nodule was detected in segment 2 under the right hepatic dome. (**b**) The left panel shows a virtual image reconstructed based on CT performed before ablation. The right panel shows a real ultrasonographic image during ablation. The circle indicates the target tumor. Left artificial pleural effusion was performed before ablation to get a clear view of the tumor. (**c**) Multiplanar reconstructed images before (left) and after (right) ablation. The tumor was completely ablated (arrow head)

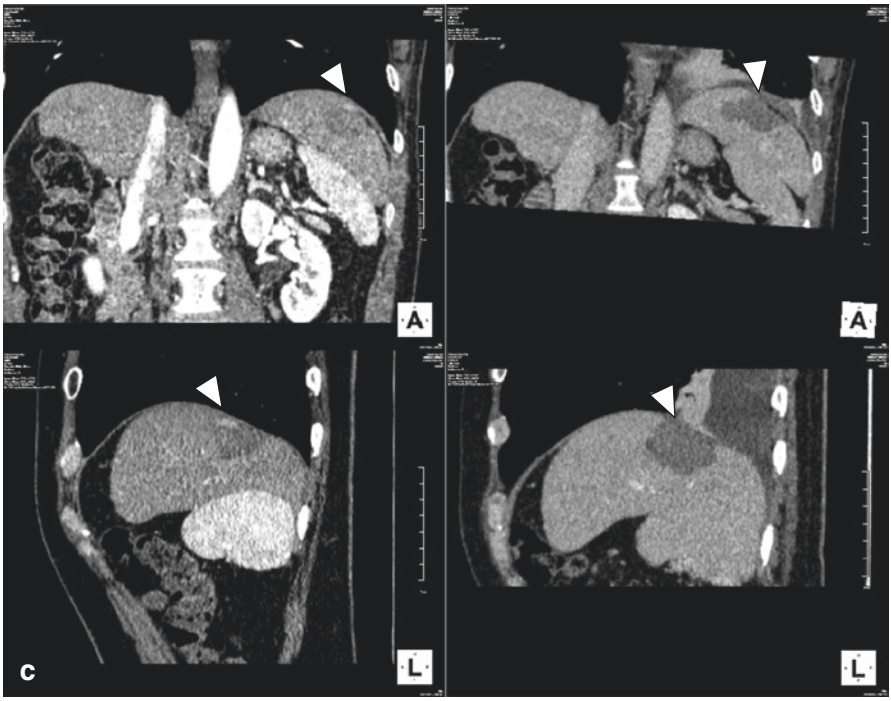

Fig. 10.7 (continued)

lesions and/or microvascular invasion near the primary lesion and may be attributable to variation in safety margin of ablation between operators [38]. Another factor is subjectivity in determining local tumor progression. Some investigators do not count tumor recurrence in adjacent area as local tumor progression when the primary site is completely ablated, following the determination of treatment failure in hepatectomy. In fact, 34.7% of patients with single HCC nodule treated with RFA had at least one recurrent nodule in the same liver subsegment of the primary lesion [39]. MWA and cryoablation are considered to be superior or at least equivalent to RFA in terms of local tumor control since they are less sensitive to the heat-sink effect [14, 18, 25, 40]. However, there is a trade-off between larger ablation zone and increased risk of complications and liver failure.

Overall Survival

Overall survival is the most important long-term outcome in the HCC treatments. RFA and ethanol injection as the initial treatment can achieve overall survival beyond 10 years [7, 22, 37, 41]. Ethanol injection has yielded 5-year survival rates of 50–60% and 10-year survival rates of approximately 20% when the 3/3 rule was

applied [22, 41]. RFA showed similar survival rates of approximately 60% at 5 years and approximately 30% at 10 years posttreatment [7, 37]. One unique feature in HCC prognosis is the frequent and repeated tumor recurrence even after complete resection or ablation of the initial primary tumor [42]. This is reflected in the characteristic survival rates that keep declining over time even in patients who had successful treatment of the initial tumors. Percutaneous ablation is a valuable modality because of its applicability to the recurrent tumors with the high local tumor control capability. Precise prediction of HCC recurrence at early stage will maximize the value of percutaneous ablation to ultimately prolong overall survival.

Percutaneous ablation and surgical resection share the same prognostic factors, e.g., tumor size and number, tumor differentiation, alpha-fetoprotein elevation, age, hepatic functional reserve, and liver disease etiology [43]. Among them, untreated chronic hepatitis is a critical factor [44]. Together with the nature of HCC prone to recur multiple times, the choice of treatment modality applied to the initial tumor has less impact on overall survival compared to the etiology, especially when the initial tumor is treated well at early stage. It also highlights importance to control liver disease etiologies such as viral hepatitis and metabolic disorders to substantially improve overall survival.

Combination with Other Treatment Modalities

Combination with Transarterial Embolization

The ablation zone by RFA or MWA can be expanded by occluding blood flow by a balloon catheter or transarterial embolization to reduce the surrounding blood flow and the heat-sink effect [45]. There are several studies, including randomized controlled trials, that compared survival of patients with medium to large HCC treated by RFA with or without transarterial chemoembolization (TACE) [46, 47]. Although the results are somewhat inconsistent, single-electrode RFA with TACE for HCC lager than 3 cm in diameter suggestively lowers local progression rate (Fig. 10.8).

Combination with Immunotherapy

Immunomodulatory effect of percutaneous ablation, either with the use of extreme heat or cold, has been investigated for decades in multiple cancer types. One of the earlier observations is spontaneous regression of untreated tumor accompanied with ablation of other tumors [48, 49]. In thermal ablation by RFA and MWA, the electrode directly heats the tissue with emitted energy in the central zone. Outside the central zone, there is transitional zone heated to 41–50 °C by thermal conduction from the central zone [50]. Inflammatory cells, including neutrophils, macrophages, dendritic cells, natural killer cells, B cells,

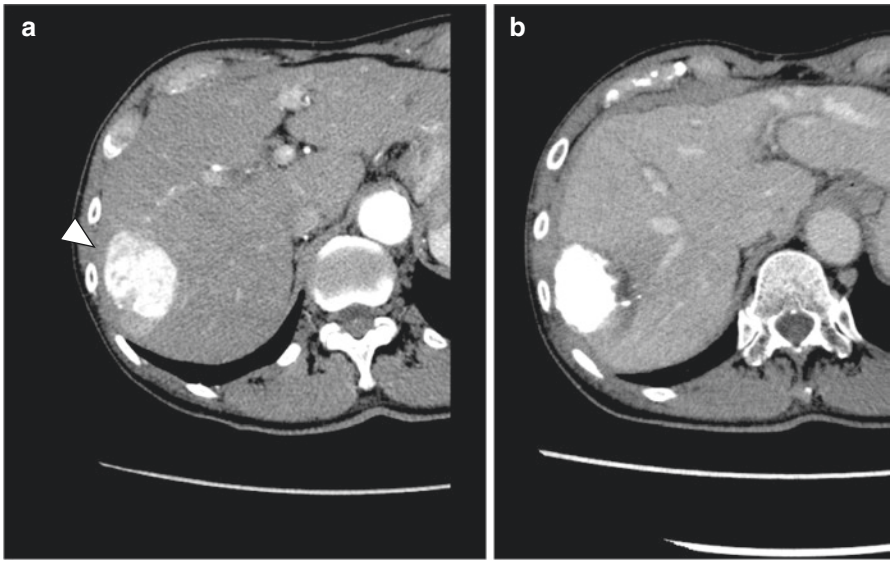

Fig. 10.8 A case with 5 cm HCC treated with TACE + RFA. (**a**) An arterial phase of dynamic CT shows hypervascular tumor in segment 7. (**b**) The tumor was treated with TACE + RFA. A dense lipiodol deposit was surrounded by un-enhanced area indicating ablative margin

and T cells, were found to infiltrate in the transitional zone, some of which may elicit tumor-specific immune response [51, 52]. These immune cells were also observed in distant unablated tumors and peripheral blood, suggesting a systemic immune response induced by thermal ablation. Similar immune activation was observed in the presence of necrosis and apoptosis induced by cryoablation [53]. More prominent release of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 was observed in cryoablation compared to heat ablation, although this may be related to cryoshock, the critical complication unique to cryoablation. Antigen accumulation in dendritic cells was also greater in cryoablation compared to RFA [54].

Although such immune reaction induced by percutaneous ablation is often observed, its clinically recognizable antitumor effect is rarely seen in daily clinical practice. This may indicate that the infiltrating immune cells need additional step(s) to be activated to elicit cancer cell killing. Recently developed immunotherapy agents, especially immune checkpoint inhibitors, may serve as drivers that activate such antitumor immununity [55]. In a pilot study combining RFA or cryoablation with tremelimumab, a monoclonal antibody to cytotoxic T-lymphocyte-associated protein 4 (CTLA4), an increase of intra-tumor CD8+ T cells was observed after 6 weeks of treatment in patients who showed response [56]. A phase III randomized controlled trial is ongoing to test nivolumab, monoclonal antibody to programmed cell death 1 (PD-1), as adjuvant therapy in patients who are at high risk of recurrence after curative HCC resection or ablation (NCT03383458).

Complications

Complications of percutaneous ablation have been well described in RFA, MWA, and ethanol injection [57]. Low-grade pain and transient fever and increased liver enzymes are commonly observed minor side effects. This section summarizes major (incidences that need specific therapy and potentially result in permanent disability or death) or minor (incidences that need no or minimal therapy including overnight admission for observation only) complications according to the grade of complications defined by the Society of Interventional Radiology [58].

Bleeding

Bleeding is a common complication across percutaneous ablation techniques that use needle-type devices especially because HCC patients often have coagulopathy due to underlying cirrhosis (Fig. 10.9). Bleeding complications are categorized as hemoperitoneum, hemothorax, and hemobilia [59]. Hemoperitoneum or intrahepatic hemorrhage is a consequence of intrahepatic vascular injury by needle devices. The risk factors of hemoperitoneum include long needle tract to the index tumor and low platelet counts [59]. The risk likely increases according to the needle thickness and number of needle insertion sessions to achieve destruction of target tumor. Bleeding is more frequent in cryoablation than RFA and MWA that have hemostatic effect per se. In fact, RFA and MWA can be used to stop bleeding by coagulating the bleeding point. Recently developed length-adjustable electrode enables the use of RFA for this purpose, with 1 cm electrode exposure.

Hemothorax is a rarer complication than hemoperitoneum and is caused by injury of intercostal arteries. However, once hemothorax occurs, the morality rate is higher than hemoperitoneum because spontaneous hemostasis less likely happens in arterial bleeding. Furthermore, bleeding to the pleural cavity causes reactive pleural effusion

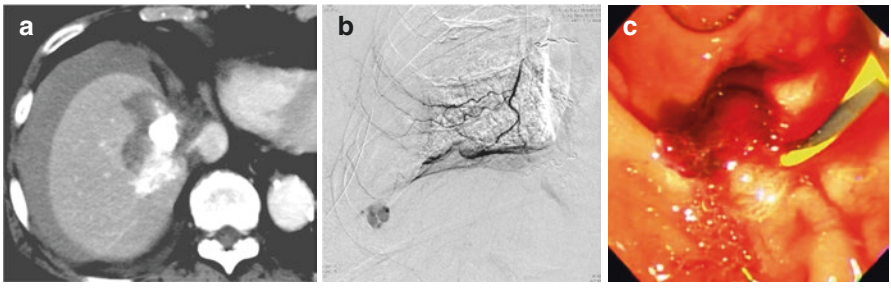

Fig. 10.9 Bleeding complications of percutaneous ablation. (**a**) Hemoperitoneum is intraperitoneal bleeding from intrahepatic vasculature. (**b**) Hemothorax is bleeding to the pleural cavity from intercostal arteries. Hemothorax is always complicated with pleural effusion and consequently respiratory impairment. (**c**) Hemobilia is bleeding from intrahepatic bile ducts where the fistula between the hepatic artery or portal vein and bile duct exists

and sometimes systematic inflammatory response, which could lead to respiratory failure. Involvement of interventional radiologist is needed to control hemothrax. When the intervention is ineffective, surgical procedures should be considered.

Hemobilia is a bleeding caused by injuring intrahepatic portal vein or artery and bile duct simultaneously with the needle devices. Unlike other two bleeding complications, hemobilia is rarely accompanied with hypovolemic shock. On the other hand, hemobilia is often first recognized as obstructive jaundice. Hemobilia can be identified by hemobilia sign, a clot formation in the gallbladder [60]. Hemobilia is generally self-limiting, and endoscopic intervention should be withheld unless patients are complicated with infection, since the intervention sometimes promotes rebleeding and infection [61].

Infection

Major infectious complications include liver abscess and cholangitis. Liver abscess is likely related to trans-biliary bacterial translocation. Previous history of biliary intervention that causes enterobiliary reflux is a strong risk factor for developing liver abscess after percutaneous ablation. Specifically, a history of enterobiliary anastomosis is a contraindication for RFA and MWA given the extremely high risk (>50%) of abscess after these procedures [62]. Cholangitis is rarer complication compared to abscess [57]. However, the incidence of cholangitis may be underestimated because the diagnosis of cholangitis is often indeterminate unless accompanied with hemobilia and obstructive jaundice.

Biliary Injury

Intrahepatic biliary injury often emerges as intrahepatic bile duct dilatation more frequently after RFA or MWA than cryoablation. Heat produced by the thermal procedures can injure the intrahepatic bile duct, where blood flow-related local cooling effect is not expected. Peripheral biliary injury is usually asymptomatic with elevated alkaline phosphatase and gamma-glutamyl transferase levels in the blood. However, injury of major intrahepatic bile duct can cause segmental atrophy of the liver parenchyma, which may lead to long-term deterioration of liver function [63]. IRE is expected to preserve biliary structure and therefore can be an alternative to RFA and MWA when the index tumor is located close to hepatic hilum [28].

Neoplastic Seeding

Neoplastic seeding is another well-documented complication in percutaneous ablation of HCC (Fig. 10.10). Intrahepatic neoplastic seeding can be surgically resected or re-ablated, but it is difficult to treat intraperitoneal dissemination of

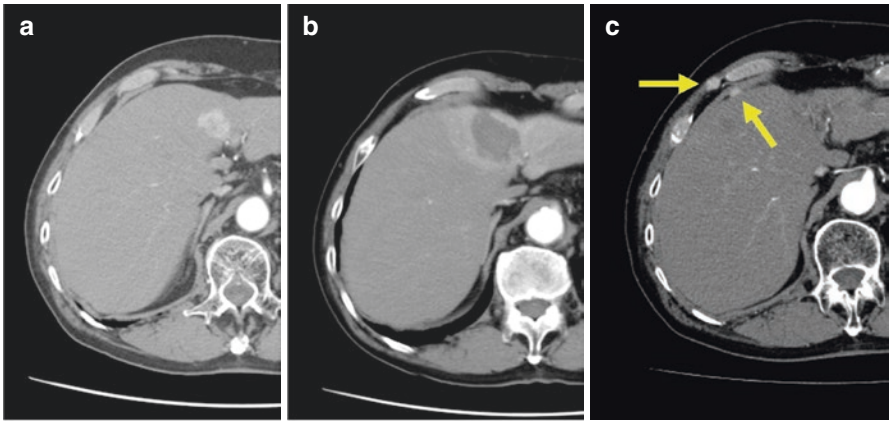

Fig. 10.10 Neoplastic seeding. (**a**) Hypervascular HCC tumor in segment 4. (**b**) The tumor was completely ablated by RFA. (**c**) Intraperitoneal tumor seeding (arrow) along the needle tract was detected 1.5 years later

cancer cells, which could significantly deteriorate patient prognosis. The risk factors of neoplastic seeding include tumor located on the surface of the liver, poorly differentiated histology, and multiple treatment sessions [64, 65]. Preceding tumor biopsy can also cause neoplastic seeding [66]. In order to avoid direct puncture of the target tumor and consequential neoplastic seeding, "no-touch" ablation using multiple electrodes inserted around the index tumor has been developed [14].

Radiotherapy

The emergence of CT-based 3D conformal radiotherapy in the 1980s enabled more precise tumor targeting with reduced radiation-induced liver injury compared to whole-liver irradiation and opened the path toward further development of radiotherapy in HCC [67]. Newly established techniques, including intensity-modulated radiotherapy (IMRT), stereotactic ablative radiotherapy (SABR) also known as stereotactic body radiation therapy (SBRT), and charged particle therapy such as proton beam therapy and carbon ion radiotherapy, have been utilized to achieve either local tumor control or enhanced efficacy of other treatment modalities.

There is certain diversity in their indication and application (e.g., does, schedule) across geographic regions, representing a range of dominant disease stages and infrastructure for radiotherapy, and high-level clinical evidence such as randomized controlled trial (RCT) is relatively limited.

Major Radiotherapeutic Approaches

Because of relative scarcity of high-level clinical evidence as well as geographic diversity in tumor characteristics and access to the technologies and facilities, there is no globally accepted indication and treatment protocols, but in general, SABR and proton beam therapy are more widely used compared to other new modalities to achieve local tumor control or to enhance efficacy of other treatment modalities such as transarterial chemoembolization, whereas 3D conformal radiotherapy is utilized when such new techniques are not available.

Stereotactic Ablative Radiotherapy (SABR)

Recent technological development has enabled development of SABR, which delivers highly conformal radiation dose within tumor, while sparing large portions of the liver from radiation-induced liver disease (RILD), and is typically indicated for treatment of inoperable tumors [68]. Although evidence supporting its survival benefit is still limited, local tumor control rates that are comparable to resection and RFA and exceeding transarterial chemoembolization have been reported (Table 10.1). SABR can be applied to treat tumors in technically less accessible and/or challenging regions such as hepatic hilum and dome. Tumors close to or invading into the vasculature can also be treated without being affected by blood flow. SABR has been applied to diverse and somewhat biased tumor stages across geographic regions, and therefore there is no commonly used protocol. For example, small HCC tumors in livers with preserved function tend to be treated with higher radiation dose in Japan, whereas more advanced tumors in functionally impaired livers are more likely treated with lower dose in Western countries and China. Predictive score or biomarker of response to SABR will enable more personalized application. Combination with medical therapies, especially immune checkpoint inhibitors, has also been explored [69].

Charged Particle Therapy

Charged particle therapy such as proton beam therapy and carbon ion therapy is characterized by its higher radiation dose concentration in tumor compared to X-ray and a high local tumor control rate comparable to SABR (Table 10.2) [70]. Application to various forms of advanced tumor such as portal venous tumor thrombus (PVTT) and large tumors (>10 cm in diameter) has been reported [71–73]. Concern about adverse event is noted when targeted lesions are located close to hepatic hilum and gastrointestinal tract, although relatively rarely observed [74–80]. Indocyanine green retention test (ICG-r15) was reported to predict posttreatment prognosis in Child-Pugh class A patients [81].

Table 10.1 Stereotactic ablative radiotherapy

Author	Study design	Indication for SABR	Combined therapy	No. of patients	HCC stage	Dose/ fraction	Local control rate	Overall survival	Adverse event[a]	Reference
Takeda et al.	Phase II	Ineligible for resection or ablation	TACE 58 (64%)	90	BCLC 0/A 86 (94%)	35–40 Gy/5fr	96.3% (3y)	66.7% (3y)	Elevated transaminase, thrombocytopenia, worsening of CPS by 2 points	[87]
Bujold et al.	Phase I/II	Inoperable	Alone	102	TNM stage III 67 (66%)	24–54 Gy/6 fr	87.0% (1y)	Median 17.0 mo	Elevated transaminase, elevated bilirubin, thrombocytopenia	[88]
Kim et al.	Phase I/II	Ineligible for resection, RFA, or TACE	Alone	32	BCLC A 31 (97%)	36–60 Gy/4 fr	25.0–94.1% (2y)	81.3% (2y)	Leukocytopenia, thrombocytopenia	[89]
Tse et al.	Phase I	Inoperable	Alone	31	AJCC stage III 19 (61%)	24–54 Gy/6 fr	NA	48.0% (1y)	Elevated transaminase, elevated bilirubin, thrombocytopenia	[90]
Cardenes et al.	Phase I	Ineligible for resection	Alone	17	T1N0 12 (70%)	36–48 Gy/3 fr	100%	60.0% (2y)	Elevated bilirubin, thrombocytopenia, elevated transaminase	[91]
Kim et al.	Phase I	Ineligible for resection	Alone	18	NA	36–60 Gy/4 fr	71.3% (2y)	69.3% (2y)	Thrombocytopenia, leukocytopenia	[92]
Sanuki et al.	Retrospective	Inoperable	Alone	185	UICC T1N0M0 156 (84%)	35–40 Gy/5 fr	91.0% (3y)	70.0% (3y)	Worsening of CPS	[93]
Su et al.	Retrospective	Ineligible for resection or ablation	Alone	132	BCLC A 73 (55%)	28–46 Gy/1–5 fr	90.9% (1y)	64.3% (5y)	Hepatic failure, GI hemorrhage	[94]

(continued)

Table 10.1 (continued)

Author	Study design	Indication for SABR	Combined therapy	No. of patients	HCC stage	Dose/ fraction	Local control rate	Overall survival	Adverse event[a]	Reference
Kuo et al.	Retrospective	Ineligible for resection or ablation	Alone	141	BCLC C 96 (68%)	26–40 Gy/3–5 fr	72–82% (3y)	33–50% (3y)	Thrombocytopenia, elevated transaminase	[95]
Teraoka et al.	Retrospective	Refusal to resection or ablation	TACE	117	BCLC 0/A 117 (100%)	40–60 Gy/4–8 fr	98.1–98.4% (3y)	63.9–67.7% (3y)	Hypoalbuminemia, thrombocytopenia, anemia	[96]
Kimura et al.	Retrospective	Ineligible for resection or ablation	TACE (81%)	150	BCLC 0/A 150 (100%)	48 Gy/4 fr	95.4–98.5% (2y)	78.6–80.3% (2y)	Thrombocytopenia	[97]
Buckstein et al.	Retrospective	Salvage or adjuvant	DEB-TACE	103	BCLC B 63 (61%)	24–50 Gy/3–5 fr	89% (2y)	Median, 23.9mo	NA	[98]

The list contains prospective studies and retrospective studies with 100 or more patients who were treated with stereotactic ablative radiotherapy
SABR stereotactic ablative radiotherapy, *HCC* hepatocellular carcinoma, *TACE* transarterial chemoembolization, *BCLC* Barcelona Clinic Liver Cancer, *fr* fraction, *CPS* Child-Pugh score, *RFA* radiofrequency ablation, *AJCC* American Joint Committee on Cancer, *UICC* Union for International Cancer Control, *DEB* drug-eluted beads

[a]Grade 3 or more adverse events are listed. When more than three types of adverse events were reported, the three most prevalent adverse events are listed

Table 10.2 Charged particle radiotherapy

Author	Study design	No. of patients	HCC stage	Dose/fraction	Local control rate	Overall survival	Adverse event[a]	Reference
Proton								
Bush	RCT (vs. TACE)	33	Milan, 23 (70%)	70.2 Gy/15 fr	88.0% (2y)	59% (2y)	Liver failure	[99]
Bush	Phase II	76	Milan, 35 (46%)	63.0 Gy/15 fr	NA	NA	None	[80]
Hong	Phase II	44	BCLC A/B, 16 (50%)	58.05–67.5 Gy/15fr	94.8% (2y)	Median 49.9 m	NA	[74]
Fukumitsu	Phase II	51	AJCC Stage I, 31 (61%)	66.0 Gy/10 fr	87.8% (5y)	38.7% (5y)	Radiation pneumonitis	[100]
Kawashima	Phase II	30	Clinical stage II, 19 (63%)	76 Gy/20 fr	96% (2y)	66.0% (2y)	Leukocytopenia, thrombocytopenia, elevated transaminase	[75]
Kim	Phase I	27	BCLC A/B/C, 13/10/4 (48/37/15%)	60–72 Gy/20–24 fr	71.4–83.3% (3y)	42.3% (5y)	None	[77]
Hong	Phase I	15	NA	45–75 Gy/15 fr	Local rec 6.7%	33.0% (3y)	Thrombocytopenia, elevated bilirubin, GI perforation, GI bleeding, fatigue	[101]
Chen	Retrospective	149	All HCCs had PVTT	Median 33 Gy (range, 5–60 Gy)	CR + PR, 35 (24%)	Median 9.4 m	NA	[102]
Fukuda	Retrospective	129	BCLC 0 or A/B/C, 30/34/65 (23/26/51%)	66–77 Gy/10–35 fr	BCLC 0 or A/B/C, 94/87/75% (5y)	Median BCLC 0 or A/B/C, 92/70/39 m.	None	[103]
Carbon ion								
Kato	Phase I/II	24	Stage II/III/IV, 10/6/8	49.5–79.5 Gy/15 fr	81.0% (3y)	25.0% (5y)	Dermatitis	[76]

(continued)

Table 10.2 (continued)

Author	Study design	No. of patients	HCC stage	Dose/fraction	Local control rate	Overall survival	Adverse event[a]	Reference
Kasuya	Phase I/II	82	BCLC A/B/C/D, 25/11/46/0	48–70 Gy/4–12 fr	89.1% (5y)	25.6% (5y)	Thrombocytopenia, worsening of CPS by 2 points, dermatitis	[104]
	Phase II	44	BCLC A/B/C/D, 9/6/29/0	52.8 Gy/4 fr	91.6% (5y)	25.0% (5y)		
Shibuya	Retrospective	174	UICC stage I/II/IIIA, 117/50/7 (67/29/4%)	48–60 Gy/2–4 fr	81.0% (3y)	73.3% (3y)	Dermatitis, myopathy, rib fracture, encephalopathy	[105]

The list contains prospective studies and retrospective studies with 100 or more patients who were treated with charged particle therapy
HCC hepatocellular carcinoma, *RCT* randomized controlled trial, *TACE* transarterial chemoembolization, *fr* fraction, *BCLC* Barcelona Clinic Liver Cancer, *AJCC* American Joint Committee on Cancer, *GI* gastrointestinal, *PVTT* portal vein tumor thrombosis, *CR* complete response, *PR* partial response, *CPS* Child-Pugh score, *UICC* Union for International Cancer Control
[a]Grade 3 or more adverse events are listed. When more than three types of adverse events were reported, the three most prevalent adverse events are listed

3D Conformal Radiotherapy

Compared to the new modalities such as SABR and charged particle therapy, it is less likely to achieve comparable irradiation dose and local tumor control with 3D conformal radiotherapy. However, given the limited availability of the new techniques, 3D conformal radiotherapy still has roles as palliative and/or supplementary therapy in daily clinical practice. Multiple prospective and retrospective studies have reported survival benefit of combining 3D conformal radiotherapy to TACE, although such clinical trials were mostly conducted in China [82, 83]. In combination with surgical resection, survival benefit was observed in patients with small (<5 cm in diameter) tumors [84]. Combination with RFA, PEI, or high-intensity focused ultrasound (HIFU) did not yield survival benefit. In patients with unresectable HCC with PVTT, TACE combined with radiotherapy yielded better survival compared to sorafenib after propensity score matching [85, 86].

Conclusions

Locoregional therapies have significantly expanded available options in the HCC treatment algorithms by providing good local tumor control with less invasive procedures compared to surgical therapies. Recent advancements in technologies and supporting techniques have contributed to substantial expansion of our capability to treat HCC at various stages. With the better characterization of the new modalities and identification of prognostic factors for each specific clinical scenario, it is expected that personalized application of the methods is facilitated to maximize prognosis of HCC patients.

References

1. European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2018;69(1):182–236. https://doi.org/10.1016/j.jhep.2018.03.019.
2. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67(1):358–80. https://doi.org/10.1002/hep.29086.
3. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017;11(4):317–70. https://doi.org/10.1007/s12072-017-9799-9.
4. Ebara M, Ohto M, Sugiura N, Kita K, Yoshikawa M, Okuda K, et al. Percutaneous ethanol injection for the treatment of small hepatocellular carcinoma. Study of 95 patients. J Gastroenterol Hepatol. 1990;5(6):616–26.
5. Mulier S, Ni Y, Jamart J, Ruers T, Marchal G, Michel L. Local recurrence after hepatic radiofrequency coagulation: multivariate meta-analysis and review of contributing factors. Ann Surg. 2005;242(2):158–71.

6. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334(11):693–9. https://doi.org/10.1056/NEJM199603143341104.
7. Shiina S, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, et al. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. Am J Gastroenterol. 2012;107(4):569–77. ; quiz 78. https://doi.org/10.1038/ajg.2011.425.
8. Goldberg SN, Gazelle GS, Solbiati L, Rittman WJ, Mueller PR. Radiofrequency tissue ablation: increased lesion diameter with a perfusion electrode. Acad Radiol. 1996;3(8):636–44.
9. Rossi S, Di Stasi M, Buscarini E, Quaretti P, Garbagnati F, Squassante L, et al. Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. AJR Am J Roentgenol. 1996;167(3):759–68.
10. Ishii H, Okada S, Nose H, Okusaka T, Yoshimori M, Takayama T, et al. Local recurrence of hepatocellular carcinoma after percutaneous ethanol injection. Cancer. 1996;77(9):1792–6. https://doi.org/10.1002/(SICI)1097-0142(19960501)77:9<1792::AID-CNCR6>3.0.CO;2-E.
11. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or =4 cm. Gastroenterology. 2004;127(6):1714–23.
12. Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology. 2005;129(1):122–30.
13. Goldberg SN, Gazelle GS, Mueller PR. Thermal ablation therapy for focal malignancy: a unified approach to underlying principles, techniques, and diagnostic imaging guidance. AJR Am J Roentgenol. 2000;174(2):323–31.
14. Hocquelet A, Aube C, Rode A, Cartier V, Sutter O, Manichon AF, et al. Comparison of no-touch multi-bipolar vs. monopolar radiofrequency ablation for small HCC. J Hepatol. 2017;66(1):67–74. https://doi.org/10.1016/j.jhep.2016.07.010.
15. Chang W, Lee JM, Lee DH, Yoon JH, Kim YJ, Yoon JH, et al. Comparison of switching bipolar ablation with multiple cooled wet electrodes and switching monopolar ablation with separable clustered electrode in treatment of small hepatocellular carcinoma: a randomized controlled trial. PLoS One. 2018;13(2):e0192173. https://doi.org/10.1371/journal.pone.0192173.
16. Seki T, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. Cancer. 1994;74(3):817–25.
17. Omata M, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: ethanol injection therapy and radiofrequency ablation. Gastroenterology. 2004;127(5 Suppl 1):S159–66.
18. Huang S, Yu J, Liang P, Yu X, Cheng Z, Han Z, et al. Percutaneous microwave ablation for hepatocellular carcinoma adjacent to large vessels: a long-term follow-up. Eur J Radiol. 2014;83(3):552–8. https://doi.org/10.1016/j.ejrad.2013.12.015.
19. Potretzke TA, Ziemlewicz TJ, Hinshaw JL, Lubner MG, Wells SA, Brace CL, et al. Microwave versus radiofrequency ablation treatment for hepatocellular carcinoma: a comparison of efficacy at a single center. J Vasc Interv Radiol. 2016;27(5):631–8. https://doi.org/10.1016/j.jvir.2016.01.136.
20. Yu J, Yu XL, Han ZY, Cheng ZG, Liu FY, Zhai HY, et al. Percutaneous cooled-probe microwave versus radiofrequency ablation in early-stage hepatocellular carcinoma: a phase III randomised controlled trial. Gut. 2017;66(6):1172–3. https://doi.org/10.1136/gutjnl-2016-312629.
21. Sugiura N, Takara K, Ohto M, Okuda K, Hirooka N. Treatment of small hepatocellular carcinoma by percutaneous injection of ethanol into tumor with real-time ultrasound monitoring. Acta Hpatol Jpn (Japanese). 1983;24:920.
22. Shiina S, Tateishi R, Imamura M, Teratani T, Koike Y, Sato S, et al. Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors. Liver Int. 2012;32(9):1434–42. https://doi.org/10.1111/j.1478-3231.2012.02838.x.

23. Mala T. Cryoablation of liver tumours -- a review of mechanisms, techniques and clinical outcome. Minim Invasive Ther Allied Technol. 2006;15(1):9–17. https://doi.org/10.1080/13645700500468268.
24. Seifert JK, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. World J Surg. 1999;23(2):109–13; discussion 13-4.
25. Ei S, Hibi T, Tanabe M, Itano O, Shinoda M, Kitago M, et al. Cryoablation provides superior local control of primary hepatocellular carcinomas of >2 cm compared with radiofrequency ablation and microwave coagulation therapy: an underestimated tool in the toolbox. Ann Surg Oncol. 2015;22(4):1294–300. https://doi.org/10.1245/s10434-014-4114-7.
26. Kim GM, Won JY, Kim MD, Park SI, Lee do Y, Shin W, et al. Cryoablation of hepatocellular carcinoma with high-risk for percutaneous ablation: safety and efficacy. Cardiovasc Intervent Radiol. 2016;39(10):1447–54. https://doi.org/10.1007/s00270-016-1384-4.
27. Davalos RV, Mir IL, Rubinsky B. Tissue ablation with irreversible electroporation. Ann Biomed Eng. 2005;33(2):223–31.
28. Seror O. Ablative therapies: advantages and disadvantages of radiofrequency, cryotherapy, microwave and electroporation methods, or how to choose the right method for an individual patient? Diagn Interv Imaging. 2015;96(6):617–24. https://doi.org/10.1016/j.diii.2015.04.007.
29. Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Inoue T, et al. Percutaneous ultrasound-guided radiofrequency ablation with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. J Gastroenterol. 2003;38(11):1066–70. https://doi.org/10.1007/s00535-003-1197-5.
30. Kondo Y, Yoshida H, Tateishi R, Shiina S, Kawabe T, Omata M. Percutaneous radiofrequency ablation of liver cancer in the hepatic dome using the intrapleural fluid infusion technique. Br J Surg. 2008;95(8):996–1004. https://doi.org/10.1002/bjs.6058.
31. Rhim H, Lim HK. Radiofrequency ablation for hepatocellular carcinoma abutting the diaphragm: the value of artificial ascites. Abdom Imaging. 2009;34(3):371–80. https://doi.org/10.1007/s00261-008-9408-4.
32. Kondo Y, Yoshida H, Shiina S, Tateishi R, Teratani T, Omata M. Artificial ascites technique for percutaneous radiofrequency ablation of liver cancer adjacent to the gastrointestinal tract. Br J Surg. 2006;93(10):1277–82. https://doi.org/10.1002/bjs.5374.
33. Minami Y, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, et al. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. AJR Am J Roentgenol. 2007;188(2):489–94. https://doi.org/10.2214/ajr.05.1286.
34. Masuzaki R, Shiina S, Tateishi R, Yoshida H, Goto E, Sugioka Y, et al. Utility of contrast-enhanced ultrasonography with Sonazoid in radiofrequency ablation for hepatocellular carcinoma. J Gastroenterol Hepatol. 2011;26(4):759–64. https://doi.org/10.1111/j.1440-1746.2010.06559.x.
35. Minami Y, Kudo M, Chung H, Inoue T, Takahashi S, Hatanaka K, et al. Percutaneous radiofrequency ablation of sonographically unidentifiable liver tumors. Feasibility and usefulness of a novel guiding technique with an integrated system of computed tomography and sonographic images. Oncology. 2007;72(Suppl 1):111–6. https://doi.org/10.1159/000111716.
36. Minami T, Minami Y, Chishina H, Arizumi T, Takita M, Kitai S, et al. Combination guidance of contrast-enhanced US and fusion imaging in radiofrequency ablation for hepatocellular carcinoma with poor conspicuity on contrast-enhanced US/fusion imaging. Oncology. 2014;87(Suppl 1):55–62. https://doi.org/10.1159/000368146.
37. Kim YS, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, et al. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. J Hepatol. 2013;58(1):89–97. https://doi.org/10.1016/j.jhep.2012.09.020.
38. Jiang C, Liu B, Chen S, Peng Z, Xie X, Kuang M. Safety margin after radiofrequency ablation of hepatocellular carcinoma: precise assessment with a three-dimensional reconstruction technique using CT imaging. Int J Hyperth. 2018;34(8):1135–41. https://doi.org/10.1080/02656736.2017.1411981.

39. Tateishi R, Shiina S, Akahane M, Sato J, Kondo Y, Masuzaki R, et al. Frequency, risk factors and survival associated with an intrasubsegmental recurrence after radiofrequency ablation for hepatocellular carcinoma. PLoS One. 2013;8(4):e59040. https://doi.org/10.1371/journal.pone.0059040.
40. Vietti Violi N, Duran R, Guiu B, Cercueil JP, Aube C, Digklia A, et al. Efficacy of microwave ablation versus radiofrequency ablation for the treatment of hepatocellular carcinoma in patients with chronic liver disease: a randomised controlled phase 2 trial. Lancet Gastroenterol Hepatol. 2018;3(5):317–25. https://doi.org/10.1016/S2468-1253(18)30029-3.
41. Ebara M, Okabe S, Kita K, Sugiura N, Fukuda H, Yoshikawa M, et al. Percutaneous ethanol injection for small hepatocellular carcinoma: therapeutic efficacy based on 20-year observation. J Hepatol. 2005;43(3):458–64. https://doi.org/10.1016/j.jhep.2005.03.033.
42. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. 2018;68(3):526–49. https://doi.org/10.1016/j.jhep.2017.09.016.
43. Tandon P, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. Liver Int. 2009;29(4):502–10. https://doi.org/10.1111/j.1478-3231.2008.01957.x.
44. Minami T, Tateishi R, Shiina S, Nakagomi R, Kondo M, Fujiwara N, et al. Comparison of improved prognosis between hepatitis B- and hepatitis C-related hepatocellular carcinoma. Hepatol Res. 2015;45(10):E99–E107. https://doi.org/10.1111/hepr.12468.
45. Kitamoto M, Imagawa M, Yamada H, Watanabe C, Sumioka M, Satoh O, et al. Radiofrequency ablation in the treatment of small hepatocellular carcinomas: comparison of the radiofrequency effect with and without chemoembolization. AJR Am J Roentgenol. 2003;181(4):997–1003. https://doi.org/10.2214/ajr.181.4.1810997.
46. Kim JW, Kim JH, Won HJ, Shin YM, Yoon HK, Sung KB, et al. Hepatocellular carcinomas 2-3 cm in diameter: transarterial chemoembolization plus radiofrequency ablation vs. radiofrequency ablation alone. Eur J Radiol. 2012;81(3):e189–93. https://doi.org/10.1016/j.ejrad.2011.01.122.
47. Peng ZW, Zhang YJ, Chen MS, Xu L, Liang HH, Lin XJ, et al. Radiofrequency ablation with or without transcatheter arterial chemoembolization in the treatment of hepatocellular carcinoma: a prospective randomized trial. J Clin Oncol. 2013;31(4):426–32. https://doi.org/10.1200/JCO.2012.42.9936.
48. Soanes WA, Ablin RJ, Gonder MJ. Remission of metastatic lesions following cryosurgery in prostatic cancer: immunologic considerations. J Urol. 1970;104(1):154–9.
49. Sanchez-Ortiz RF, Tannir N, Ahrar K, Wood CG. Spontaneous regression of pulmonary metastases from renal cell carcinoma after radio frequency ablation of primary tumor: an in situ tumor vaccine? J Urol. 2003;170(1):178–9. https://doi.org/10.1097/01.ju.0000070823.38336.7b.
50. Chu KF, Dupuy DE. Thermal ablation of tumours: biological mechanisms and advances in therapy. Nat Rev Cancer. 2014;14(3):199–208. https://doi.org/10.1038/nrc3672.
51. Wissniowski TT, Hansler J, Neureiter D, Frieser M, Schaber S, Esslinger B, et al. Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. Cancer Res. 2003;63(19):6496–500.
52. Dromi SA, Walsh MP, Herby S, Traughber B, Xie J, Sharma KV, et al. Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. Radiology. 2009;251(1):58–66. https://doi.org/10.1148/radiol.2511072175.
53. Seifert JK, France MP, Zhao J, Bolton EJ, Finlay I, Junginger T, et al. Large volume hepatic freezing: association with significant release of the cytokines interleukin-6 and tumor necrosis factor a in a rat model. World J Surg. 2002;26(11):1333–41. https://doi.org/10.1007/s00268-002-6139-5.
54. den Brok MH, Sutmuller RP, Nierkens S, Bennink EJ, Frielink C, Toonen LW, et al. Efficient loading of dendritic cells following cryo and radiofrequency ablation in combination with immune modulation induces anti-tumour immunity. Br J Cancer. 2006;95(7):896–905. https://doi.org/10.1038/sj.bjc.6603341.

55. Shi L, Chen L, Wu C, Zhu Y, Xu B, Zheng X, et al. PD-1 blockade boosts radiofrequency ablation-elicited adaptive immune responses against tumor. Clin Cancer Res. 2016;22(5):1173–84. https://doi.org/10.1158/1078-0432.CCR-15-1352.
56. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. J Hepatol. 2017;66(3):545–51. https://doi.org/10.1016/j.jhep.2016.10.029.
57. Bertot LC, Sato M, Tateishi R, Yoshida H, Koike K. Mortality and complication rates of percutaneous ablative techniques for the treatment of liver tumors: a systematic review. Eur Radiol. 2011;21(12):2584–96. https://doi.org/10.1007/s00330-011-2222-3.
58. Ahmed M, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, et al. Image-guided tumor ablation: standardization of terminology and reporting criteria--a 10-year update. J Vasc Interv Radiol. 2014;25(11):1691–705 e4. https://doi.org/10.1016/j.jvir.2014.08.027.
59. Goto E, Tateishi R, Shiina S, Masuzaki R, Enooku K, Sato T, et al. Hemorrhagic complications of percutaneous radiofrequency ablation for liver tumors. J Clin Gastroenterol. 2010;44(5):374–80. https://doi.org/10.1097/MCG.0b013e3181b7ed76.
60. Obi S, Shiratori Y, Shiina S, Hamamura K, Kato N, Imamura M, et al. Early detection of haemobilia associated with percutaneous ethanol injection for hepatocellular carcinoma. Eur J Gastroenterol Hepatol. 2000;12(3):285–90.
61. Sasahira N, Tada M, Yoshida H, Tateishi R, Shiina S, Hirano K, et al. Extrahepatic biliary obstruction after percutaneous tumour ablation for hepatocellular carcinoma: aetiology and successful treatment with endoscopic papillary balloon dilatation. Gut. 2005;54(5):698–702. https://doi.org/10.1136/gut.2003.038331.
62. Iida H, Aihara T, Ikuta S, Yamanaka N. Risk of abscess formation after liver tumor radiofrequency ablation: a review of 8 cases wtih a history of enterobiliary anastomosis. Hepato-Gastroenterology. 2014;61(135):1867–70.
63. Kondo Y, Shiina S, Tateishi R, Arano T, Uchino K, Enooku K, et al. Intrahepatic bile duct dilatation after percutaneous radiofrequency ablation for hepatocellular carcinoma: impact on patient's prognosis. Liver Int. 2011;31(2):197–205. https://doi.org/10.1111/j.1478-3231.2010.02415.x.
64. Imamura J, Tateishi R, Shiina S, Goto E, Sato T, Ohki T, et al. Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. Am J Gastroenterol. 2008;103(12):3057–62. https://doi.org/10.1111/j.1572-0241.2008.02153.x.
65. Francica G. Needle track seeding after radiofrequency ablation for hepatocellular carcinoma: prevalence, impact, and management challenge. J Hepatocell Carcinoma. 2017;4:23–7. https://doi.org/10.2147/JHC.S106558.
66. Livraghi T, Lazzaroni S, Meloni F, Solbiati L. Risk of tumour seeding after percutaneous radiofrequency ablation for hepatocellular carcinoma. Br J Surg. 2005;92(7):856–8. https://doi.org/10.1002/bjs.4986.
67. Park SH, Kim JC, Kang MK. Technical advances in external radiotherapy for hepatocellular carcinoma. World J Gastroenterol. 2016;22(32):7311–21. https://doi.org/10.3748/wjg.v22.i32.7311.
68. Meyer J, Singal AG. Stereotactic ablative radiotherapy for hepatocellular carcinoma: history, current status, and opportunities. Liver Transpl. 2018;24(3):420–7. https://doi.org/10.1002/lt.24991.
69. Keane FK, Hong TS, Zhu AX. Evolving systemic therapy in hepatocellular carcinoma: current management and opportunities for integration with radiotherapy. Semin Radiat Oncol. 2018;28(4):332–41. https://doi.org/10.1016/j.semradonc.2018.06.006.
70. Igaki H, Mizumoto M, Okumura T, Hasegawa K, Kokudo N, Sakurai H. A systematic review of publications on charged particle therapy for hepatocellular carcinoma. Int J Clin Oncol. 2018;23(3):423–33. https://doi.org/10.1007/s10147-017-1190-2.
71. Lee SU, Park JW, Kim TH, Kim YJ, Woo SM, Koh YH, et al. Effectiveness and safety of proton beam therapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis. Strahlenther Onkol. 2014;190(9):806–14. https://doi.org/10.1007/s00066-014-0604-6.

72. Sugahara S, Nakayama H, Fukuda K, Mizumoto M, Tokita M, Abei M, et al. Proton-beam therapy for hepatocellular carcinoma associated with portal vein tumor thrombosis. Strahlenther Onkol. 2009;185(12):782–8. https://doi.org/10.1007/s00066-009-2020-x.
73. Sugahara S, Oshiro Y, Nakayama H, Fukuda K, Mizumoto M, Abei M, et al. Proton beam therapy for large hepatocellular carcinoma. Int J Radiat Oncol Biol Phys. 2010;76(2):460–6. https://doi.org/10.1016/j.ijrobp.2009.02.030.
74. Hong TS, Wo JY, Yeap BY, Ben-Josef E, McDonnell EI, Blaszkowsky LS, et al. Multi-institutional Phase II study of high-dose hypofractionated proton beam therapy in patients with localized, unresectable hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Clin Oncol. 2016;34(5):460–8. https://doi.org/10.1200/JCO.2015.64.2710.
75. Kawashima M, Furuse J, Nishio T, Konishi M, Ishii H, Kinoshita T, et al. Phase II study of radiotherapy employing proton beam for hepatocellular carcinoma. J Clin Oncol. 2005;23(9):1839–46. https://doi.org/10.1200/JCO.2005.00.620.
76. Kato H, Tsujii H, Miyamoto T, Mizoe JE, Kamada T, Tsuji H, et al. Results of the first prospective study of carbon ion radiotherapy for hepatocellular carcinoma with liver cirrhosis. Int J Radiat Oncol Biol Phys. 2004;59(5):1468–76. https://doi.org/10.1016/j.ijrobp.2004.01.032.
77. Kim TH, Park JW, Kim YJ, Kim BH, Woo SM, Moon SH, et al. Phase I dose-escalation study of proton beam therapy for inoperable hepatocellular carcinoma. Cancer Res Treat. 2015;47(1):34–45. https://doi.org/10.4143/crt.2013.218.
78. Hong TS, Ryan DP, Borger DR, Blaszkowsky LS, Yeap BY, Ancukiewicz M, et al. A phase 1/2 and biomarker study of preoperative short course chemoradiation with proton beam therapy and capecitabine followed by early surgery for resectable pancreatic ductal adenocarcinoma. Int J Radiat Oncol Biol Phys. 2014;89(4):830–8. https://doi.org/10.1016/j.ijrobp.2014.03.034.
79. Abei M, Okumura T, Fukuda K, Hashimoto T, Araki M, Ishige K, et al. A phase I study on combined therapy with proton-beam radiotherapy and in situ tumor vaccination for locally advanced recurrent hepatocellular carcinoma. Radiat Oncol. 2013;8:239. https://doi.org/10.1186/1748-717X-8-239.
80. Bush DA, Kayali Z, Grove R, Slater JD. The safety and efficacy of high-dose proton beam radiotherapy for hepatocellular carcinoma: a phase 2 prospective trial. Cancer. 2011;117(13):3053–9. https://doi.org/10.1002/cncr.25809.
81. Mizumoto M, Oshiro Y, Okumura T, Fukuda K, Fukumitsu N, Abei M, et al. Association between pretreatment retention rate of indocyanine green 15 min after administration and life prognosis in patients with HCC treated by proton beam therapy. Radiother Oncol. 2014;113(1):54–9. https://doi.org/10.1016/j.radonc.2014.08.015.
82. Zou LQ, Zhang BL, Chang Q, Zhu FP, Li YY, Wei YQ, et al. 3D conformal radiotherapy combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. World J Gastroenterol. 2014;20(45):17227–34. https://doi.org/10.3748/wjg.v20.i45.17227.
83. Cho JY, Paik YH, Park HC, Yu JI, Sohn W, Gwak GY, et al. The feasibility of combined transcatheter arterial chemoembolization and radiotherapy for advanced hepatocellular carcinoma. Liver Int. 2014;34(5):795–801. https://doi.org/10.1111/liv.12445.
84. Yu W, Wang W, Rong W, Wang L, Xu Q, Wu F, et al. Adjuvant radiotherapy in centrally located hepatocellular carcinomas after hepatectomy with narrow margin (<1 cm): a prospective randomized study. J Am Coll Surg. 2014;218(3):381–92. https://doi.org/10.1016/j.jamcollsurg.2013.11.030.
85. Kim GA, Shim JH, Yoon SM, Jung J, Kim JH, Ryu MH, et al. Comparison of chemoembolization with and without radiation therapy and sorafenib for advanced hepatocellular carcinoma with portal vein tumor thrombosis: a propensity score analysis. J Vasc Interv Radiol. 2015;26(3):320–9 e6. https://doi.org/10.1016/j.jvir.2014.10.019.
86. Wang K, Guo WX, Chen MS, Mao YL, Sun BC, Shi J, et al. Multimodality treatment for hepatocellular carcinoma with portal vein tumor thrombus: a large-scale, multicenter, propensity mathching score analysis. Medicine (Baltimore). 2016;95(11):e3015. https://doi.org/10.1097/MD.0000000000003015.

87. Takeda A, Sanuki N, Tsurugai Y, Iwabuchi S, Matsunaga K, Ebinuma H, et al. Phase 2 study of stereotactic body radiotherapy and optional transarterial chemoembolization for solitary hepatocellular carcinoma not amenable to resection and radiofrequency ablation. Cancer. 2016;122(13):2041–9. https://doi.org/10.1002/cncr.30008.
88. Bujold A, Massey CA, Kim JJ, Brierley J, Cho C, Wong RK, et al. Sequential phase I and II trials of stereotactic body radiotherapy for locally advanced hepatocellular carcinoma. J Clin Oncol. 2013;31(13):1631–9. https://doi.org/10.1200/JCO.2012.44.1659.
89. Kim JW, Kim DY, Han KH, Seong J. Phase I/II trial of helical IMRT-based stereotactic body radiotherapy for hepatocellular carcinoma. Dig Liver Dis. 2018; https://doi.org/10.1016/j.dld.2018.11.004.
90. Tse RV, Hawkins M, Lockwood G, Kim JJ, Cummings B, Knox J, et al. Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Clin Oncol. 2008;26(4):657–64. https://doi.org/10.1200/JCO.2007.14.3529.
91. Cardenes HR, Price TR, Perkins SM, Maluccio M, Kwo P, Breen TE, et al. Phase I feasibility trial of stereotactic body radiation therapy for primary hepatocellular carcinoma. Clin Transl Oncol. 2010;12(3):218–25. https://doi.org/10.1007/s12094-010-0492-x.
92. Kim JW, Seong J, Lee IJ, Woo JY, Han KH. Phase I dose escalation study of helical intensity-modulated radiotherapy-based stereotactic body radiotherapy for hepatocellular carcinoma. Oncotarget. 2016;7(26):40756–66. https://doi.org/10.18632/oncotarget.9450.
93. Sanuki N, Takeda A, Oku Y, Mizuno T, Aoki Y, Eriguchi T, et al. Stereotactic body radiotherapy for small hepatocellular carcinoma: a retrospective outcome analysis in 185 patients. Acta Oncol. 2014;53(3):399–404. https://doi.org/10.3109/0284186X.2013.820342.
94. Su TS, Liang P, Lu HZ, Liang J, Gao YC, Zhou Y, et al. Stereotactic body radiation therapy for small primary or recurrent hepatocellular carcinoma in 132 Chinese patients. J Surg Oncol. 2016;113(2):181–7. https://doi.org/10.1002/jso.24128.
95. Kuo HT, Que J, Lin LC, Yang CC, Koay LB, Lin CH. Impact of tumor size on outcome after stereotactic body radiation therapy for inoperable hepatocellular carcinoma. Medicine (Baltimore). 2017;96(50):e9249. https://doi.org/10.1097/MD.0000000000009249.
96. Teraoka Y, Kimura T, Aikata H, Daijo K, Osawa M, Honda F, et al. Clinical outcomes of stereotactic body radiotherapy for elderly patients with hepatocellular carcinoma. Hepatol Res. 2018;48(2):193–204. https://doi.org/10.1111/hepr.12916.
97. Kimura T, Aikata H, Doi Y, Imano N, Takeuchi Y, Takahashi I, et al. Comparison of stereotactic body radiation therapy combined with or without transcatheter arterial chemoembolization for patients with small hepatocellular carcinoma ineligible for resection or ablation therapies. Technol Cancer Res Treat. 2018;17:1533033818783450. https://doi.org/10.1177/1533033818783450.
98. Buckstein M, Kim E, Fischman A, Blacksburg S, Facciuto M, Schwartz M, et al. Stereotactic body radiation therapy following transarterial chemoembolization for unresectable hepatocellular carcinoma. J Gastrointest Oncol. 2018;9(4):734–40. https://doi.org/10.21037/jgo.2018.05.01.
99. Bush DA, Smith JC, Slater JD, Volk ML, Reeves ME, Cheng J, et al. Randomized clinical trial comparing proton beam radiation therapy with transarterial chemoembolization for hepatocellular carcinoma: results of an interim analysis. Int J Radiat Oncol Biol Phys. 2016;95(1):477–82. https://doi.org/10.1016/j.ijrobp.2016.02.027.
100. Fukumitsu N, Sugahara S, Nakayama H, Fukuda K, Mizumoto M, Abei M, et al. A prospective study of hypofractionated proton beam therapy for patients with hepatocellular carcinoma. Int J Radiat Oncol Biol Phys. 2009;74(3):831–6. https://doi.org/10.1016/j.ijrobp.2008.10.073.
101. Hong TS, DeLaney TF, Mamon HJ, Willett CG, Yeap BY, Niemierko A, et al. A prospective feasibility study of respiratory-gated proton beam therapy for liver tumors. Pract Radiat Oncol. 2014;4(5):316–22. https://doi.org/10.1016/j.prro.2013.10.002.

102. Chen MY, Wang YC, Wu TH, Lee CF, Wu TJ, Chou HS, et al. Efficacy of external beam radiation-based treatment plus locoregional therapy for hepatocellular carcinoma associated with portal vein tumor thrombosis. Biomed Res Int. 2016;2016:6017406. https://doi.org/10.1155/2016/6017406.
103. Fukuda K, Okumura T, Abei M, Fukumitsu N, Ishige K, Mizumoto M, et al. Long-term outcomes of proton beam therapy in patients with previously untreated hepatocellular carcinoma. Cancer Sci. 2017;108(3):497–503. https://doi.org/10.1111/cas.13145.
104. Kasuya G, Kato H, Yasuda S, Tsuji H, Yamada S, Haruyama Y, et al. Progressive hypo-fractionated carbon-ion radiotherapy for hepatocellular carcinoma: combined analyses of 2 prospective trials. Cancer. 2017;123(20):3955–65. https://doi.org/10.1002/cncr.30816.
105. Shibuya K, Ohno T, Terashima K, Toyama S, Yasuda S, Tsuji H, et al. Short-course carbon-ion radiotherapy for hepatocellular carcinoma: a multi-institutional retrospective study. Liver Int. 2018;38(12):2239–47. https://doi.org/10.1111/liv.13969.

Chapter 11
Molecular-Targeted Therapies in Hepatocellular Carcinoma

Amit Mahipal, Anuhya Kommalapati, Rutika Mehta, and Richard D. Kim

Introduction

Liver cancers are expected to account for approximately 42, 000 new cases and 30,000 deaths in 2018 in the United States, 90% of which are hepatocellular carcinoma (HCC) [1]. The incidence of the disease has almost tripled since mid-1980s and HCC-related cancer deaths are increasing at a rapid pace as compared to other cancer types. Early-stage HCC (stage 1 and some stage II cancers) is generally treated with surgery and in selected cases with liver transplantation. In contrast, the majority of patients present with unresectable, advanced HCC, and require locoregional therapy (ablation, arterially directed therapies, or external beam radiation therapy) or systemic treatment depending on the extent of the disease and their functional status [2]. Systemic treatment options typically include molecular-targeted therapy with sorafenib or enrollment into clinical trials. Sorafenib had been the only FDA-approved therapy for systemic treatment of advanced HCC for almost a decade. More recently, other systemic therapies such as regorafenib and nivolumab have been approved as second-line treatment for patients with HCC who progress on sorafenib [2]. In addition, other molecular targeted agents such as lenvatinib, pembrolizumab, and cabozantinib have shown promising results in recent clinical trials [3–6]. With broader range of systemic therapies becoming available, the treatment of patients with HCC now involves a decision-making process with consideration for the magnitude of the beneficial effects of the therapeutic agent as

A. Mahipal
Department of Medical Oncology, Mayo Clinic, Rochester, MN, USA

A. Kommalapati
Department of Internal Medicine, University of South Carolina School of Medicine, Columbia, SC, USA

R. Mehta · R. D. Kim (✉)
Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA
e-mail: richard.kim@moffitt.org

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_11

well as other factors including adverse events, patient baseline functional status, comorbidities, and Child-Pugh score. Clinical trials with new active agents are in progress, and many of these trials focused on targeted therapies and immunotherapies with the intense interest in developing novel, more effective, and less toxic agents. The current chapter details about the molecular pathogenesis of HCC, targeted therapies, and newer systemic therapies on the horizon in the management of HCC. For a better understanding of molecular-targeted therapies for HCC, we detailed some of the key signaling pathway alterations that play a significant role in the pathogenesis of HCC. A detailed description of immunotherapy and related pathways in the management of advanced HCC in described elsewhere in Chap. 12.

Molecular Therapeutic Targets in HCC

Activation of receptor tyrosine kinase (RTK) by inducing the RAS-MAPK/ERK and PI3K-Akt kinase signaling pathways is observed generally in less than 5% of HCC tumors [7]. Phosphorylation of RTKs such as vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor (HGFR)/c-MET, and the stem cell growth factor receptor (c-kit) leads to activation of the MAPK and PI3K pathways. This activation of MAPK/ERK pathway triggers the proto-oncogene cFos and transcription factor AP-1/c-Jun, thereby leading to tumor cell proliferation [8]. In approximately half of the HCC cases, activation of PI3K-AKT kinase pathway via insulin/insulin-like growth factor (IGF) receptors result in mTOR activation, thereby promoting the carcinogenesis [8]. Loss of function of *PTEN*, a tumor suppressor gene by mutation or epigenetic silencing, can also lead to the activation of PI3K-AKT kinase pathway [7]. In addition, polymorphism of *EGF* gene results in the triggering of EGFR pathway, thereby leading to HCC initiation. A case-control study has shown that hepatic expression-associated *EGF* gene polymorphism (SNP rs44449030) (G/G versus A/A) in cirrhotic patients results in fourfold increase in the risk of HCC [9].

FGFR pathway is another RTK pathway that has been implicated in the pathogenesis of HCC. The ligands of FGF family interact with four FGF receptors (FGFR1–4). Among these four subtypes of receptors, FGFR4 is the most abundant receptor expressed in hepatocytes. The ligand, FGF19, binds to FGFR4 and plays an important role in regulating bile acid synthesis and hepatocyte proliferation. The activation of this FGF19-FGFR4 pathway activation may play a key role in a proportion of HCCs, and this pathway is a potential therapeutic target [8]. A phase I clinical trial that evaluated a highly selective small molecular FGFR4 inhibitor showed promising results in advanced HCC tumors that harbored FGF19-activated tumors. The maximum tolerated dose was 600 mg. This study opened new therapeutic approach in the management of advanced HCC that express FGF19.

Another RTK pathway implicated in the pathogenesis of HCC is the HGF (ligand)/c-MET pathway. Activation of c-MET triggers the stimulation of

downstream effector molecules, PI3K and ERK. A subgroup analysis in patients in a randomized trial evaluating sorafenib revealed an increased expression of HGF levels in patients who did not respond to sorafenib [10]. These high HGF levels correlated with poor prognosis. It is postulated that *MET* activation was associated with vascular invasion and poor prognosis in human HCC [10].

HCC is a highly vascular tumor and neo-angiogenesis is a prominent feature, with the hepatic artery as the major source of its blood supply. VEGF and other vascular growth factors (PDGF, FGF) play a prominent role in promoting and sustaining neo-angiogenesis in HCC [11]. This neo-angiogenesis from pre-existing vasculature is a fundamental process of the supply of oxygen and nutrients to the expanding tumor mass. The increased expression of both VEGF and its receptor (VEGFR) is frequently seen in HCC, and VEGF level correlates with microvessel density, angiogenic activity, tumor progression, metastasis, postoperative recurrence, and poor prognosis [12]. A cross talk between VEGF and FGF also plays a key role in the angiogenesis of HCC. The expression of FGFR, VEGFR and PDGFR, and their ligands are increased in HCC, and high expression of FGFR is also related to the capsular invasion of neoplastic cells. In addition, PDGF overexpression has been linked to the increased metastatic potential of HCC. It is important to note that the increased VEGF expression in the tumor mass often leads to disorganized and immature neo-vascularization. This immature neo-angiogenesis can lead to focal areas of tumor hypoxia in the rapidly expanding tumor mass. This hypoxic environment leads to the stimulation of growth factors such as hypoxia-inducible factor (HIF)-1 and 2 that are further shown to cause local tumor advancement and metastases, especially by increasing the expression of *SERPINB3* [13]. The increased expression of HIFs can potentially lead to failure of localized therapies such as trans-arterial embolization and poor prognosis after the treatment [8]. HIF-2 alpha antagonists that showed promising results in renal cell carcinoma may be a potential option in HCC by targeting the HIF pathway [14].

Other potential trigger of the carcinogenic tyrosine kinase pathway is the effects on the affinity of heparan sulfate for heparan sulfate-binding receptor tyrosine ligands. Many RTKs use heparan sulfate as a co-receptor, and a pair of heparan sulfate sulfatases, SULF1 and SULF2, have been shown to modulate HCC carcinogenesis and tumorigenesis [15]. In addition, increased expression of *HDAC2* gene that is involved in histone modification was found to be expressed more in HCC patients. *HDAC2* gene regulates histone deacetylases (HDACs), chromatin-modifying enzymes that are involved in epigenetic regulation of cell cycle, differentiation, and apoptosis.

The JAK-STAT signaling pathway consists of three main components: a cell surface receptor, a Janus kinase (JAK), and two signal transducer and activator of transcription (STAT) proteins [16]. A variety of interleukins, cytokines, and growth factors can trigger STATs via tyrosine phosphorylation by JAKs. Dysregulation of JAK-STAT cascade can lead to cell migration and differentiation, thereby causing cancers and immunodeficiency syndromes. This activation of JAK-STAT cascade has been implicated in the carcinogenesis of HCC [17].

Genetic mutations that alter the WNT/β-catenin signaling pathway have been implicated in the carcinogenesis in about half of the HCC cases [8]. Though multiple mechanisms that activate Wnt pathway are proposed, the most common mutations acting on the WNT/β-catenin signaling pathway are acting mutations in *CTNNB1* (results in the stabilization of β-catenin), inactivation of *APC* tumor suppressor gene, and mutations in *AXIN1* and *AXIN 2* (negative regulators of the Wnt pathway) [18]. The molecular pathogenesis in the HCC is thought to be different in different etiological entities. For example, activation of canonical WNT signaling pathway without *CTNNB1* mutations is preferentially seen in HCC tumors with more aggressive molecular and clinical features [19].

Transforming growth factor (TGF)-β pathway dysregulation, which can be caused by genetic and other types of aberrations, has been implicated in molecular pathogenesis in HCC [20]. TGF-β signaling has been shown to play a critical role in cellular proliferation, apoptosis, differentiation, motility, lineage specificity, and stem cell homeostasis. TGF-β receptor inhibitor, Galunisertib, has been tested in clinical trials as single agent or in combination with immune checkpoint inhibitors (ClinicalTrials.gov) [21].

Therapeutic intervention toward some of these pathways is now feasible by using clinically developed and/or FDA-approved agents as summarized below.

Clinically Tested Molecular-Targeted Agents for HCC

Multiple small molecule inhibitors, therapeutic antibodies, and immune checkpoint inhibitors have been evaluated in clinical trials, some of which have been approved or are soon to be approved as the first- and second-line therapies for advanced HCC (Table 11.1). Several agents such as sunitinib and tivantinib failed to meet the primary endpoint in "all-comer" clinical trials without prior patient selection. These agents may be found still beneficial in a subset of HCC patients if predictive biomarkers of response are identified.

Table 11.1 Molecular-targeted agents approved or soon-to-be-approved for advanced HCC treatment

Agent	Targets	Indication	Reference
Sorafenib	RAF, VEGFR1–3, PDGFR, c-kit	First-line	[26]
Lenvatinib	VEGFR1–3, FGFR1–4, PDGFR, RET, c-kit	First-line	[36]
Regorafenib	VEGFR1–3, PDGFR, FGFR, c-kit, RET, RAF	Second-line	[35]
Cabozantinib	c-MET, VEGFR2, Axl, RET	Second-line	[6]
Ramucirumab	VEGFR2	Second-line (AFP $\geq$ 400 ng/mL)	[38]
Nivolumab	PD-1	Second-line	[54]
Pembrolizumab	PD-1	Second-line	[55]

Sorafenib

Sorafenib, a multi-kinase (serine, threonine, and tyrosine kinases) inhibitor was the first agent that showed benefit in prolonging median overall survival (OS) in advanced HCC. The drug primarily acts by targeting receptor tyrosine kinase pathway by blocking rapidly accelerated fibrosarcoma (RAF) kinases; VEGFR1, 2, and 3; PDGFR; and c-kit. Apart from blocking RTK pathway, other potential mechanisms of action have also been postulated. A study by *Tai* et al. has shown that sorafenib also acts by targeting the STAT3 pathway [22]. The drug also targets matrix metalloproteinase-2 and Ki-67 expression via simultaneous upregulation of p53 and suppression of transcription factor, forkhead box M1 (FOXM1) [23]. Blocking of FOXM1 leads to cell-cycle arrest by causing mitotic spindle defects and chromosome disaggregation. Sorafenib is also shown to target IGF1-mediated neoplastic cell proliferation and macrophage-mediated tumor cell growth [24].

Though the mechanisms underlying the sorafenib effect are not fully understood, the drug showed prolonging median OS patients with advanced, metastatic HCC in two randomized phase III trials (SHARP and Asia Pacific Trial) [25]. SHARP trial was a multicenter randomized placebo control study involving 602 treatment-naïve advanced HCC patients. Study subjects were randomized to receive sorafenib 400 mg twice daily or placebo. The subjects in the sorafenib arm had a better median OS as compared to that of placebo arm (10.7 vs 7.9 months) (hazard ratio [HR] =0.69 [95% CI = 0.55–0.87]; $p = 0.001$). Time to radiologic progression of the disease was improved by 3 months in sorafenib arm (5.5 vs 2.8 months, $p < 0.001$). Similar results with sorafenib were seen in the Asia-Pacific study (median OS: 6.5 vs. 4.2 months, HR = 0.68 [95% CI = 0.53–0.93], $p = 0.01$; median time to progression: 2.8 vs 1.4 months, HR 0.57 [95% CI = 0.42–0.79], $p = 0.0005$). The side effect profile of sorafenib was similar in both the trials, and most common adverse events noted were weight loss, diarrhea, hand-foot skin reaction, and hypophosphatemia. Overall objective response rates (ORRs) were low, occurring in 1% of the patients, and none of the patients achieved complete response in both the trials. Both the studies included patients with good performance status of ECOG 0 or 1 (90%) and good liver function (95% of patients in sorafenib group were Child-Pugh class A and 5% were class B). Nonetheless, given the prolonged OS in sorafenib group, the FDA approved this medication for advanced metastatic HCC. The safety profile of sorafenib is further evaluated in a phase IV Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafenib (GIDEON) trial to evaluate safety profile and drug efficacy in various patient subgroups [26].

Sorafenib was also evaluated in a phase II double-blinded randomized trial in combination with doxorubicin involving 96 advanced HCC survivors. The study subjects were randomized to doxorubicin alone or in combination with sorafenib. The combination group showed significant improvement in time to progression (6.4 vs. 2.8 months; $p = 0.02$), progression-free survival (PFS) (6 vs. 2.7 months; $p = 0.006$), and median OS (13.7 vs. 6.7 months; $p = 0.006$) [27]. However, these

positive results were not replicated in phase III trial and the study was suspended prematurely due to increased toxicity and unlikely advantages of the combination regimen [28].

Sorafenib was also evaluated in combination with local therapies such as transarterial chemoembolization (TACE). TACE is generally reserved as a palliative treatment option for patients with locally advanced and unresectable HCC. TACE showed improved survival as compared to that of control arm that received symptomatic management only. Wang et al. showed that there is an increased expression of VEGF and PDGF after TACE procedure, which may contribute to tumor neo-angiogenesis and progression [29]. Phase III trials of combining sorafenib to TACE, TACTICS (in Japan) and TACE-2 (in UK), have been recently conducted [30–33]. In the TACTICS trial, the treatment was well tolerated and resulted in significant improvement in time to tumor progression (24.1 vs. 13.5 months; $p = 0.004$) and PFS (25.2 vs. 13.5 months; $p = 0.006$). On the other hand, TACE-2 trial showed no significant improvement in PFS (326 vs. 320 days; HR = 1.01; 95% CI = 0.78–1.30; $p = 0.94$) and OS (631 vs. 598 days; HR = 0.91 [95% CI 0.67–1.24]; $p = 0.57$) by adding sorafenib to TACE. Sorafenib was also evaluated in the adjuvant setting after surgical resection in phase II and III trails, which showed mixed results. A recent meta-analysis reported no significant benefit of using sorafenib as postsurgical adjuvant therapy [34].

Regorafenib

Regorafenib, structurally related to sorafenib, was approved as a second-line agent for the use in HCC patients whose disease progressed while on sorafenib. Regorafenib is also multikinase inhibitor blocking the tyrosine kinase pathway, thereby targeting the angiogenesis (VEGFR1, 2 and 3), tumor environment (PDGFR and FGFR), and oncogenesis (c-kit, RET, and RAF). Regorafenib was evaluated in an open-label, phase II trial involving 36 HCC patients with Barcelona Clinic Liver Cancer stage B or C HCC and preserved to mildly impaired liver function (Child-Pugh class A) who progressed on sorafenib therapy. Median time to progression (TTP) was 4.3 months and median OS was 13.8 months. Most common adverse events noted were hand-foot syndrome, fatigue, hypertension, diarrhea, and hypothyroidism. The drug was further evaluated in a phase III RESORCE trial that randomized 573 patients with HCC into best supportive care plus either regorafenib 160 mg once daily (3 weeks on/1 week off) arm or placebo arm ($n = 194$) [35]. All study subjects were on prior sorafenib therapy with a documented radiological progression. The regorafenib group showed significant improvement in median OS (10.6 vs. 7.8 months; HR = 0.63; 95% CI: 0.50–0.79; $p < 0.0001$), median TTP (3.2 vs. 1.5 months; HR = 0.44; 95% CI = 0.036–0.55; $p < 0.001$), and PFS (3.1 vs. 1.5 months; HR = 0.46; 95% CI = 0.37–0.56; $p < 0.001$) [35]. Patients in regorafenib arm had a better overall response rate (10.6% vs. 4.1%; $p = 0.005$) and overall disease control rate (65.2% vs. 36.1%; $p < 0.001$) as compared to that of placebo.

Most common adverse events noted in regorafenib group were hypertension, hand-foot skin reaction, fatigue, and diarrhea.

Lenvatinib

Lenvatinib is a small molecule inhibitor of multiple kinases, VEGFR1–3, FGFR1–4, PDGFR, RET, and c-kit, which was approved as a first-line therapy for advanced HCC. In a phase II trial that involved 46 metastatic HCC patients, lenvatinib-treated patients had a median OS of 18.7 months with a median TTP of 12.8 months. Though none of the patients demonstrated complete response, 47% of the patients had stable disease, and subgroup analyses showed similar promising results. Lenvatinib was subsequently evaluated in an open-label noninferiority phase III REFLECT trial [36]. Median PFS (7.4 vs. 3.7 months; HR = 0.66; 95% CI = 0.57–0.77; $p < 0.0001$) and median TTP (8.9 vs. 3.7 months; HR = 0.63; 95% CI = 0.53–0.73; $p < 0.0001$) favored lenvatinib over sorafenib. Lenvatinib was demonstrated to be noninferior to sorafenib in terms of median OS (13.6 vs. 12.3 months; HR = 0.92; 95% CI = 0.79–1.06), which was the primary endpoint. Most common adverse events noted in the lenvatinib arm were hypertension (41–76%), hand-foot erythrodysesthesia syndrome (65%), proteinuria (61%), diarrhea (39%), and fatigue (30–61%). Of note, 57% of the patients experienced severer adverse events (grade 3 and above) with 18% of the patients reporting treatment-related serious adverse events.

Cabozantinib

Cabozantinib is an oral kinase pathway inhibitor, targeting c-MET, VEGFR2, Axl, and RET, which has been evaluated in clinical trials, enrolling advanced HCC patients [4, 6]. In a phase II trial that evaluated cabozantinib in 41 advanced HCC patients, partial response or stable disease was observed in 66% of the patients. Median PFS in cabozantinib and placebo groups were 2.5 and 1.4 months, respectively (difference was not statistically significant). In a randomized double-blind placebo-controlled phase III CELESTIAL trial involving 707 advanced HCC patients (2:1 randomization), cabozantinib was associated with 24% reduced risk of dying as compared to that of placebo group, and median OS improved by 2.2 months (10.2 vs. 8.0 months; HR = 0.76; 95% CI = 0.63–0.92; $p = 0.0049$) [6]. Cabozantinib was also associated with a better median PFS (5.2 vs. 1.9 months; HR: 0.44, 95% CI = 0.36–0.52; $p < 0.0001$). Most common adverse events noted in cabozantinib group were diarrhea (20%), palmar-plantar erythrodysesthesia (15%), and thrombocytopenia (15%). Grade 5 toxicities (hepatic failure, esophagobronchial fistula, portal vein thrombosis, upper gastrointestinal hemorrhage, pulmonary embolism, and hepatorenal syndrome) were seen in six patients who received cabozantinib.

Ramucirumab

Ramucirumab is a human IgG1 monoclonal antibody against VEGFR2 that has shown encouraging results in advanced HCC by targeting endothelial cell proliferation and migration. In a phase II trial involving 42 advanced HCC patients, ramucirumab was associated with a median overall survival of 12 months with 9.5% OS rate. Given these encouraging results, the drug was evaluated in a randomized placebo control phase III REACH trial, enrolling unselected 643 patients advanced HCC patients [37]. A potential OS benefit was suggested in patients with baseline AFP levels of 400 ng/mL or more and Child-Pugh score of 5 (HR = 0.61; 95% CI = 0.43–0.87; p = 0.01) and Child-Pugh score of 6 (HR = 0.64; 95% CI = 0.42–0.98; p = 0.04) in posthoc subgroup analyses. To validate the finding, a phase III REACH-2 study was conducted by enrolling 292 advanced HCC patients with baseline AFP 400 ng/mL or greater and Child-Pugh class A who progressed on or were intolerant to sorafenib (NCT02435433) [38]. Patients were randomized (2:1) to receive ramucirumab 8 mg/kg i.v. or placebo. Ramucirumab treatment significantly improved median OS (8.5 vs. 7.3 months; HR = 0.71; 95% CI = 0.53–0.95; p = 0.02) and PFS (2.8 vs. 1.6 months; HR = 0.45; 95% CI = 0.34–0.60; $p < 0.0001$). Risk of death was reduced by 29%. ORRs were 4.6% and 1.1% (p = 0.12) and disease control rates (ORR + stable disease) were 59.9% and 38.9% (p = 0.0006) in ramucirumab and placebo arms, respectively. Adverse events (grade 3 and above) occurred include hypertension (12.2% in ramucirumab and 5.3% in placebo) and hyponatremia (5.6% in ramucirumab and 0% in placebo). REACH-2 is the first positive phase III study conducted in biomarker-selected patients with HCC.

Sunitinib

Sunitinib is an oral multikinase inhibitor that targets VEGFR1–3, PDGFR, c-kit, and RET. In phase II trials in advanced HCC patients, ORRs were 2.7–12% and median Oss were 5.8–9.8 months [39]. With the high dose of sunitinib (50 mg daily, 4 weeks on and 2 weeks off) in these trials, high incidences of grade 3 or 4 toxicities, such as thrombocytopenia, neutropenia, asthenia, hand-foot syndrome, and fatal treatment-related events, were noted. In a phase III trial, reduced dose of sunitinib (37.5 mg daily for 4 weeks on and 2 weeks off) was compared to sorafenib 400 mg twice daily in 1074 Child-Pugh class A patients with advanced HCC [40]. The trial was terminated prematurely due to drug-related toxicity and inferior outcomes in sunitinib arm. Patients in the sunitinib arm had a shorter median OS compared to that of sorafenib arm (7.9 vs. 10.2 months; HR = 1.30; 95% CI = 1.13–1.30; p = 0.001).

Tivantinib

A small-molecule compound, tivantinib, has been tested as a c-Met inhibitor for second-line treatment of advanced HCC in a phase II trial, in which positive c-Met immunostaining was associated with extended TTP in a posthoc subgroup analysis (2.7 vs. 1.4 months; HR = 0.43; 95% CI = 0.19–0.97; p = 0.03) [41]. In the subsequent phase III METIV-HCC trial enrolling c-Met-positive inoperable HCC patients, the prognostic benefit was not validated [42]. More recent studies suggest that tivantinib is not a specific c-Met inhibitor [43].

Bevacizumab

Bevacizumab, a humanized monoclonal antibody targeting VEGF, elicits antiangiogenic effect and has been evaluated in advanced HCC patients either as a monotherapy or in combination with other agents, including erlotinib, gemcitabine with oxaliplatin, and capecitabine with or without oxaliplatin [44]. As a single agent, it showed ORR of 13% with a median OS of 12.4 months [45]. As a combination therapy with gemcitabine and oxaliplatin, the drug showed an ORR of 20% and PFS of 9.6 months [44]. Most trials of bevacizumab reported median PFS between 5.3 and 9 months and OS between 5.9 and 13.7 months with the disease control rate ranging from 51% to 77%. Most common grade 3/4 adverse events noted were increased liver enzymes (13%), fatigue (12%), high blood pressure (10%), diarrhea (8%), and neutropenia (5%). Trials of bevacizumab combined with other agents are currently under investigation in HCC (ClinicalTrials.gov).

Other Tyrosine Kinase Inhibitors

Other tyrosine kinase pathway inhibitors have been evaluated in advanced HCC patients with no positive results. Cediranib, a VEGFR, c-kit, and PDGFR inhibitor, showed no clinically significant improvement in response to therapy with a high incidence of adverse events [46]. Linifanib is an inhibitor of VEGFR and PDGFR, and has shown promising results in phase I and II trials, although it failed to show superiority to sorafenib in a phase III randomized clinical trial [47]. Moreover, linifanib was not tolerated well with high incidence of grade 3/4 adverse events leading to its discontinuation to be compared with sorafenib [47]. Similar findings were seen in phase II and III clinical trials of brivanib, an inhibitor of VEGFR and FGFR, in patients with advanced HCC [48]. In phase III randomized

sorafenib-controlled clinical trial, brivanib was found to be noninferior to sorafenib but was associated with high degree of toxicities with less tolerability profile compared to sorafenib [48]. Most common adverse events noted were hypertension, hyponatremia, and fatigue. Similarly, orantinib, an inhibitor of VEGFR2, PDGFR, and FGFR, yielded an ORR of 8.6% in a phase II clinical trial involving metastatic HCC patients [49]. The drug did not show any benefit in phase III randomized placebo-controlled trial when added as an adjuvant therapy to TACE [50].

Everolimus

Everolimus is an inhibitor of mechanistic target of rapamycin (mTOR). Despite encouraging prolongation of median OS (7.7 vs. 5.7 months) and PFS (3.7 vs. 1.9 months) compared to placebo in a phase II clinical trial [51], subsequent phase III EVOLVE I trial, enrolling 546 advanced HCC patients who did not tolerate or progressed on sorafenib, failed to validate the results for median OS (7.6 vs. 7.3 months for treatment and placebo arms, respectively) and TTP (3 vs. 2.6 months for treatment and placebo arms, respectively). Everolimus was also tested as combination therapy with sorafenib compared with sorafenib alone in a randomized phase II trial. The trial did not show any additional benefit over sorafenib monotherapy in terms of ORR (0% vs. 10%) and PFS (68% vs. 70%) with increased incidence of adverse events [52].

Future Directions

HCC is the most common type of liver cancer, and its incidence has almost tripled since 1980, and the management of advanced HCC will remain the major clinical challenge [53]. Recent approval of multiple systemic therapies has opened the avenue toward tailored medical treatment of advanced HCC according to associated specific molecular aberrations. Molecular targeted agents, which failed to demonstrate clinical benefit in unselected "all-comer" trials may still have value in a subset of patients if predictive biomarkers of response are identified. For biomarker exploration, acquisition of biospecimens, especially tissue, will be critical and inter- and intratumor heterogeneity of molecular aberrations (covered in Chap. 14) need to be addressed to elucidate clinically actionable information that guide the systemic therapies. Circulating biomarkers (covered in Chap. 7) will enable flexible clinical application of such predictive biomarkers of drug response. Given the complex molecular dysregulations implicated in HCC pathogenesis, combination therapies especially with immune-oncology agents may enable improved management of the patients with advanced HCC.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30. https://doi.org/10.3322/caac.21387.
2. (NCCN) NCCN. NNCN Guidelines version 1.2018 Hepatocellular carcinoma. 2017. https://www.nccn.org/professionals/physician_gls/pdf/hepatobiliary.pdf. Accessed 24 Feb 2018.
3. Cheng A-L, Finn RS, Qin S, Han K-H, Ikeda K, Piscaglia F, et al. Phase III trial of lenvatinib (LEN) vs sorafenib (SOR) in first-line treatment of patients (pts) with unresectable hepatocellular carcinoma (uHCC). J Clin Oncol. 2017;35(15_suppl):4001. https://doi.org/10.1200/JCO.2017.35.15_suppl.4001.
4. Kelley RK, Verslype C, Cohn AL, Yang TS, Su WC, Burris H, et al. Cabozantinib in hepatocellular carcinoma: results of a phase 2 placebo-controlled randomized discontinuation study. Ann Oncol. 2017;28(3):528–34. https://doi.org/10.1093/annonc/mdw651.
5. Finn RS, Chan SL, Zhu AX, Knox JJ, Cheng A-L, Siegel AB, et al. KEYNOTE-240: randomized phase III study of pembrolizumab versus best supportive care for second-line advanced hepatocellular carcinoma. J Clin Oncol. 2017;35(4_suppl):TPS503-TPS. https://doi.org/10.1200/JCO.2017.35.4_suppl.TPS503.
6. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. N Engl J Med. 2018;379(1):54–63. https://doi.org/10.1056/NEJMoa1717002.
7. Shibata T, Arai Y, Totoki Y. Molecular genomic landscapes of hepatobiliary cancer. Cancer Sci. 2018;109(5):1282–91. https://doi.org/10.1111/cas.13582.
8. Dhanasekaran R, Bandoh S, Roberts LR. Molecular pathogenesis of hepatocellular carcinoma and impact of therapeutic advances. F1000Res. 2016;5:F1000 Faculty Rev-879. https://doi.org/10.12688/f1000research.6946.1.
9. Tanabe KK, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA. 2008;299(1):53–60. https://doi.org/10.1001/jama.2007.65.
10. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res. 2013;19(9):2310–8. https://doi.org/10.1158/1078-0432.CCR-12-2791.
11. Bocca C, Novo E, Miglietta A, Parola M. Angiogenesis and fibrogenesis in chronic liver diseases. Cell Mol Gastroenterol Hepatol. 2015;1(5):477–88. https://doi.org/10.1016/j.jcmgh.2015.06.011.
12. Dai L, Peng XX, Tan EM, Zhang JY. Tumor-associated antigen CAPERalpha and microvessel density in hepatocellular carcinoma. Oncotarget. 2016;7(13):16985–95. https://doi.org/10.18632/oncotarget.7707.
13. Cannito S, Turato C, Paternostro C, Biasiolo A, Colombatto S, Cambieri I, et al. Hypoxia up-regulates SERPINB3 through HIF-2alpha in human liver cancer cells. Oncotarget. 2015;6(4):2206–21. https://doi.org/10.18632/oncotarget.2943.
14. Tella SH, Taieb D, Pacak K. HIF-2alpha: Achilles' heel of pseudohypoxic subtype paraganglioma and other related conditions. Eur J Cancer. 2017;86:1–4. https://doi.org/10.1016/j.ejca.2017.08.023.
15. Lai JP, Thompson JR, Sandhu DS, Roberts LR. Heparin-degrading sulfatases in hepatocellular carcinoma: roles in pathogenesis and therapy targets. Future Oncol. 2008;4(6):803–14. https://doi.org/10.2217/14796694.4.6.803.
16. Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. Science. 2002;296(5573):1653–5. https://doi.org/10.1126/science.1071545.
17. Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology. 2006;130(4):1117–28. https://doi.org/10.1053/j.gastro.2006.01.006.

18. Pez F, Lopez A, Kim M, Wands JR, Caron de Fromentel C, Merle P. Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. J Hepatol. 2013;59(5):1107–17. https://doi.org/10.1016/j.jhep.2013.07.001.
19. Goossens N, Sun X, Hoshida Y. Molecular classification of hepatocellular carcinoma: potential therapeutic implications. Hepat Oncol. 2015;2(4):371–9. https://doi.org/10.2217/hep.15.26.
20. Rao S, Mishra L. Targeting TGF-beta signaling in liver cancer. Hepatology. 2018; https://doi.org/10.1002/hep.30426.
21. Fabregat I, Caballero-Diaz D. Transforming growth factor-beta-induced cell plasticity in liver fibrosis and hepatocarcinogenesis. Front Oncol. 2018;8:357. https://doi.org/10.3389/fonc.2018.00357.
22. Tai WT, Cheng AL, Shiau CW, Huang HP, Huang JW, Chen PJ, et al. Signal transducer and activator of transcription 3 is a major kinase-independent target of sorafenib in hepatocellular carcinoma. J Hepatol. 2011;55(5):1041–8. https://doi.org/10.1016/j.jhep.2011.01.047.
23. Wei JC, Meng FD, Qu K, Wang ZX, Wu QF, Zhang LQ, et al. Sorafenib inhibits proliferation and invasion of human hepatocellular carcinoma cells via up-regulation of p53 and suppressing FoxM1. Acta Pharmacol Sin. 2015;36(2):241–51. https://doi.org/10.1038/aps.2014.122.
24. Sprinzl MF, Puschnik A, Schlitter AM, Schad A, Ackermann K, Esposito I, et al. Sorafenib inhibits macrophage-induced growth of hepatoma cells by interference with insulin-like growth factor-1 secretion. J Hepatol. 2015;62(4):863–70. https://doi.org/10.1016/j.jhep.2014.11.011.
25. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34. https://doi.org/10.1016/S1470-2045(08)70285-7.
26. Lencioni R, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, et al. GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and of its treatment with sorafeNib): second interim analysis. Int J Clin Pract. 2014;68(5):609–17. https://doi.org/10.1111/ijcp.12352.
27. Abou-Alfa GK, Johnson P, Knox JJ, Capanu M, Davidenko I, Lacava J, et al. Doxorubicin plus sorafenib vs doxorubicin alone in patients with advanced hepatocellular carcinoma: a randomized trial. JAMA. 2010;304(19):2154–60. https://doi.org/10.1001/jama.2010.1672.
28. Abou-Alfa GK, Niedzwieski D, Knox JJ, Kaubisch A, Posey J, Tan BR, et al. Phase III randomized study of sorafenib plus doxorubicin versus sorafenib in patients with advanced hepatocellular carcinoma (HCC): CALGB 80802 (Alliance). J Clin Oncol. 2016;34(4_suppl):192. https://doi.org/10.1200/jco.2016.34.4_suppl.192.
29. Wang B, Xu H, Gao ZQ, Ning HF, Sun YQ, Cao GW. Increased expression of vascular endothelial growth factor in hepatocellular carcinoma after transcatheter arterial chemoembolization. Acta Radiol. 2008;49(5):523–9. https://doi.org/10.1080/02841850801958890.
30. Chao Y, Chung YH, Han G, Yoon JH, Yang J, Wang J, et al. The combination of transcatheter arterial chemoembolization and sorafenib is well tolerated and effective in Asian patients with hepatocellular carcinoma: final results of the START trial. Int J Cancer. 2015;136(6):1458–67. https://doi.org/10.1002/ijc.29126.
31. Kudo M, Ueshima K, Ikeda M, Torimura T, Tanabe N, Aikata H, Izumi N, Yamasaki T, Nojiri S, TACTICS trial group. Randomized, open label, multicenter, phase II trial comparing transarterial chemoembolization (TACE) plus sorafenib with TACE alone in patients with hepatocellular carcinoma (HCC): TACTICS trial. ASCO, ASCO Gastrointestinal symposium. J Clin Oncol. 2018;36(suppl 4S; abstr 206):2018. Accessed 25 Feb 2018.
32. Meyer T, Fox R, Ma YT, Ross PJ, James MW, Sturgess R, et al. Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): a randomised placebo-controlled, double-blind, phase 3 trial. Lancet Gastroenterol Hepatol. 2017;2(8):565–75. https://doi.org/10.1016/S2468-1253(17)30156-5.
33. de Jesus VHF, Dettino ALA. Update on hepatocellular carcinoma from the 2018 Gastrointestinal Cancer Symposium (ASCO GI). J Hepatocell Carcinoma. 2018;5:87–90. https://doi.org/10.2147/JHC.S171396.

34. Shang J, Xu S, Zhang J, Ran X, Bai L, Tang H. Efficacy of sorafenib in patients with hepatocellular carcinoma after resection: a meta-analysis. Oncotarget. 2017;8(65):109723–31. https://doi.org/10.18632/oncotarget.21299.
35. Bruix J, Qin S, Merle P, Granito A, Huang Y-H, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 389(10064):56–66. https://doi.org/10.1016/S0140-6736(16)32453-9.
36. Kudo M, Finn RS, Qin S, Han K-H, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. https://doi.org/10.1016/S0140-6736(18)30207-1.
37. Zhu AX, Baron AD, Malfertheiner P, Kudo M, Kawazoe S, Pezet D, et al. Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma: analysis of REACH trial results by Child-Pugh score. JAMA Oncol. 2016; https://doi.org/10.1001/jamaoncol.2016.4115.
38. Zhu AX, Kang Y, Yen C, Finn RS, Galle PR, Llovet JM, et al. REACH-2: a randomized, double-blind, placebo-controlled phase 3 study of ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma (HCC) and elevated baseline alpha-fetoprotein (AFP) following first-line sorafenib. J Clin Oncol. 2018;36((15) supp)
39. Barone C, Basso M, Biolato M, Pompili M, Rufini V, Miele L, et al. A phase II study of sunitinib in advanced hepatocellular carcinoma. Dig Liver Dis. 2013;45(8):692–8. https://doi.org/10.1016/j.dld.2013.01.002.
40. Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, et al. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol. 2013;31(32):4067–75. https://doi.org/10.1200/JCO.2012.45.8372.
41. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol. 2013;14(1):55–63. https://doi.org/10.1016/S1470-2045(12)70490-4.
42. Rimassa L, Assenat E, Peck-Radosavljevic M, Pracht M, Zagonel V, Mathurin P, et al. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. Lancet Oncol. 2018;19(5):682–93. https://doi.org/10.1016/S1470-2045(18)30146-3.
43. Rebouissou S, La Bella T, Rekik S, Imbeaud S, Calatayud AL, Rohr-Udilova N, et al. Proliferation markers are associated with MET expression in hepatocellular carcinoma and predict tivantinib sensitivity in vitro. Clin Cancer Res. 2017;23(15):4364–75. https://doi.org/10.1158/1078-0432.CCR-16-3118.
44. Zhu AX, Blaszkowsky LS, Ryan DP, Clark JW, Muzikansky A, Horgan K, et al. Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24(12):1898–903. https://doi.org/10.1200/jco.2005.04.9130.
45. Siegel AB, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol. 2008;26(18):2992–8. https://doi.org/10.1200/jco.2007.15.9947.
46. Zhu AX, Ancukiewicz M, Supko JG, Sahani DV, Blaszkowsky LS, Meyerhardt JA, et al. Efficacy, safety, pharmacokinetics, and biomarkers of cediranib monotherapy in advanced hepatocellular carcinoma: a phase II study. Clin Cancer Res. 2013;19(6):1557–66. https://doi.org/10.1158/1078-0432.ccr-12-3041.
47. Cainap C, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, et al. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. J Clin Oncol. 2015;33(2):172–9. https://doi.org/10.1200/JCO.2013.54.3298.
48. Johnson PJ, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results

from the randomized phase III BRISK-FL study. J Clin Oncol. 2013;31(28):3517–24. https://doi.org/10.1200/jco.2012.48.4410.
49. Kanai F, Yoshida H, Tateishi R, Sato S, Kawabe T, Obi S, et al. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. Cancer Chemother Pharmacol. 2011;67(2):315–24. https://doi.org/10.1007/s00280-010-1320-2.
50. Kudo M, Cheng A-L, Park J-W, Park JH, Liang P-C, Hidaka H, et al. Orantinib versus placebo combined with transcatheter arterial chemoembolisation in patients with unresectable hepatocellular carcinoma (ORIENTAL): a randomised, double-blind, placebo-controlled, multicentre, phase 3 study. Lancet Gastroenterol Hepatol. 3(1):37–46. https://doi.org/10.1016/S2468-1253(17)30290-X.
51. Shiah HS, Chen CY, Dai CY, Hsiao CF, Lin YJ, Su WC, et al. Randomised clinical trial: comparison of two everolimus dosing schedules in patients with advanced hepatocellular carcinoma. Aliment Pharmacol Ther. 2013;37(1):62–73. https://doi.org/10.1111/apt.12132.
52. Koeberle D, Dufour JF, Demeter G, Li Q, Ribi K, Samaras P, et al. Sorafenib with or without everolimus in patients with advanced hepatocellular carcinoma (HCC): a randomized multicenter, multinational phase II trial (SAKK 77/08 and SASL 29). Ann Oncol. 2016;27(5):856–61. https://doi.org/10.1093/annonc/mdw054.
53. Singal AG, Murphy CC. Hepatocellular carcinoma: a roadmap to reduce incidence and future burden. J Natl Cancer Inst. 2018; https://doi.org/10.1093/jnci/djy184.
54. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017; https://doi.org/10.1016/s0140-6736(17)31046-2.
55. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018; https://doi.org/10.1016/S1470-2045(18)30351-6.

Chapter 12
Immune Therapies

Zachary J. Brown and Tim F. Greten

Introduction

Hepatocellular carcinoma (HCC) often develops in the setting of chronic liver disease and as such is often considered an inflammation-induced cancer where this inflammation aids to drive carcinogenesis [1]. As an inflammation-induced cancer, patients with a greater lymphocyte density in HCC tumors often correlate with a better prognosis [2]. As a result, immunotherapy may provide an ideal approach of treatment [3]. As of 2017, nivolumab, a monoclonal antibody targeting programmed cell death-1 (PD-1), has been approved for patients with advanced HCC in the second-line setting for those patients who have progressed on sorafenib [4]. In order to have an effective immunotherapy, a tumor must present antigens which are recognized by the immune system, and then the immune system must be able to mount a response against that tumor-associated antigen. However, the tumor microenvironment has adapted ways to evade immune recognition as well as escape from immune therapies. In this chapter, we describe immune-based treatment of HCC first focusing on HCC tumor antigens and liver tumor microenvironment. We will then discuss the application of immune-based therapies in the treatment of patients with advanced HCC as well as future directions of where the field of immunotherapy may be heading in patients with HCC.

Z. J. Brown · T. F. Greten (✉)
Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
e-mail: tim.greten@nih.gov

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_12

Tumor Antigens

The identification of HCC tumor antigens is an important step for potential targeted immune-based therapies. Solid tumors may contain 30–70 mutations that alter the amino acid sequences of proteins. Each of these alterations is foreign to the immune system and is therefore a potential target for immune recognition [5]. However, the immune system cannot recognize all mutations for an effective immune response. The mutation must result in a protein that is expressed by the tumor cell, and this protein must be able to be presented to the immune system on a human leukocyte antigen (HLA) protein [5]. If either of these conditions are not met, the mutation may not be immunogenic.

Tumor antigens can be categorized into three broad categories: tumor-specific antigens, which arise in cancer cells and are completely absent from normal cells; tumor-associated antigens (TAA) which are expressed mainly on tumor cells but also at low levels on normal cells; and cancer/testis antigens which are expressed by tumor cells and expressed in reproductive tissue but otherwise absent in normal adult cells [6]. TAA-specific $CD8^+$ T cell responses have been found to be more detectable in patients with early-stage HCCs as compared to later stages. In addition, patients with TAA-specific responses were found to have a significantly greater median progression-free survival (PFS) compared to patients without TAA-specific responses [7]. Therefore, we can deduce that tumors, which contain more immunogenic antigens tend to have a more favorable prognosis and outcome.

Perhaps the most well-known HCC antigen is alpha-fetoprotein (AFP). AFP is widely considered the most useful biomarker for HCC evaluation with high levels correlating with the development and progression of HCC [8]. AFP is present during fetal development but is largely absent in healthy adults. Other widely studied tumor antigens associated with HCC include glypican-3 (GPC-3), melanoma-associated gene-A1 (MAGE-A1), New York-esophageal squamous cell carcinoma-1 (NY-ESO-1), sarcoma X breakpoint 2 (SSX2), and telomerase reverse transcriptase (hTERT) [1, 7]. Targeting tumor antigens may be an efficient way to control tumor growth, but immune-based approaches focusing on vaccines, cytokines, and nonspecific T cell activation have resulted in largely disappointing results [1, 9]. These immune-based strategies may produce disappointing results as the HCC tissue microenvironment may have a large impact in response to therapy.

Tumor Environment

The tumor microenvironment (TME) is becoming increasingly recognized as having a role in tumor growth promotion and immune evasion. Under normal conditions, the liver experiences a tremendous antigen exposure as a result of portal-venous blood flow with physiologic filtration of environmental and bacterial agents from the gastrointestinal tract. Therefore, to prevent autoimmune damage from constant immune stimulation and antigen exposure, the liver has developed intrinsic tolerogenic mechanisms in the innate and adaptive immune responses. This

tolerance to the large flux of antigens can be harmless in respect to the large majority of antigens but may prove to be detrimental with immune tolerance to tumor-associated antigens and HCC progression [10].

In addition to the physiologic immune tolerance of the liver, chronic inflammation promotes immune suppression through continuous cytokine production and recruitment of immune cells [10]. In order to have T cell activation, $CD4^+$ T cells are presented an antigen on major histocompatibility complex (MHC) class II molecules by antigen-presenting cells that recognize a specific T cell receptor (TCR). A costimulatory binding of CD28 on the T cell with CD80 or CD86 on the antigen presenting cell (APC) is then required to propagate the signal for T cell activation. Activated $CD4^+$ T cells are then polarized toward a T_H1 phenotype enhancing $CD8^+$ T cell cytotoxicity toward the target antigen (Fig. 12.1). However, once $CD4^+$ T cells are activated, they upregulate the immunosuppressive receptor, cytotoxic T lymphocyte-associated protein (CTLA)-4, to act as a break on the adaptive immune response by competing with CD28 for the binding of CD80/CD86 and therefore taking away the necessary costimulatory signal. CTLA-4 is present on activated T cells, dendritic cells (DCs), and is constitutively expressed on regulatory T cells (Tregs). Under physiologic conditions, immune checkpoints prevent over activation of T cells, thereby limit unwanted collateral tissue damage [11]. However, the TME has usurped this physiological function to produce an immunosuppressive milieu.

In addition to CTLA-4, PD-1 also acts as a check on the immune response. Much like CTLA-4, PD-1 is upregulated in the setting of chronic antigen exposure. PD-1 is expressed by activated $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, B cells, as well as Tregs, myeloid-derived suppressor cells (MDSCs), monocytes, and DCs. Programmed death-ligand 1 (PD-L1) and PD-L2 are the ligands of PD-1. PD-L1 is found on APCs, MDSCs, macrophages, various parenchymal cells, as well as tumor cells, while PD-L2 is only expressed by hematopoietic stem cells. The binding of PD-1 with its ligand blocks TCR signaling, inhibits T cell proliferation, and leads to dysfunctional exhausted T cells [1]. Additionally, PD-L1 overexpression in HCC is associated with more aggressive tumors and an increase in postoperative recurrence of HCC [12].

Different immune cells have been reported to suppress antitumor immunity in HCC. Tregs have been shown to accumulate in patients with HCC where an increase in Tregs has been linked to a worse outcome [13]. Tregs inhibit the immune response through competitively binding CTLA-4 to CD28 as well as downregulation of CD80 and CD86, secretion of transforming growth factor (TGF)-β and IL-10, and depletion of IL-2. MDSCs are also found to be increased in patients with HCC and elevated counts often correlate to tumor progression [14–16]. MDSCs are a heterogeneous group of immature and immunosuppressive myeloid cells which promote tumor formation by facilitating angiogenesis through VEGF production, impair $CD4^+$ and $CD8^+$ T cells with increased arginase activity, impair NK cell activity via TGF-β, disrupt TCR signaling through reactive oxygen and nitrogen species, and promote Treg expansion. Additionally, MDSCs promote formation of immunosuppressive M2 macrophages which creates a vicious cycle further propagating an immunosuppressive microenvironment through production of suppressive cytokines and promoting T cells to a T_H2 phenotype which further induces M2 macrophages and MDSCs [1].

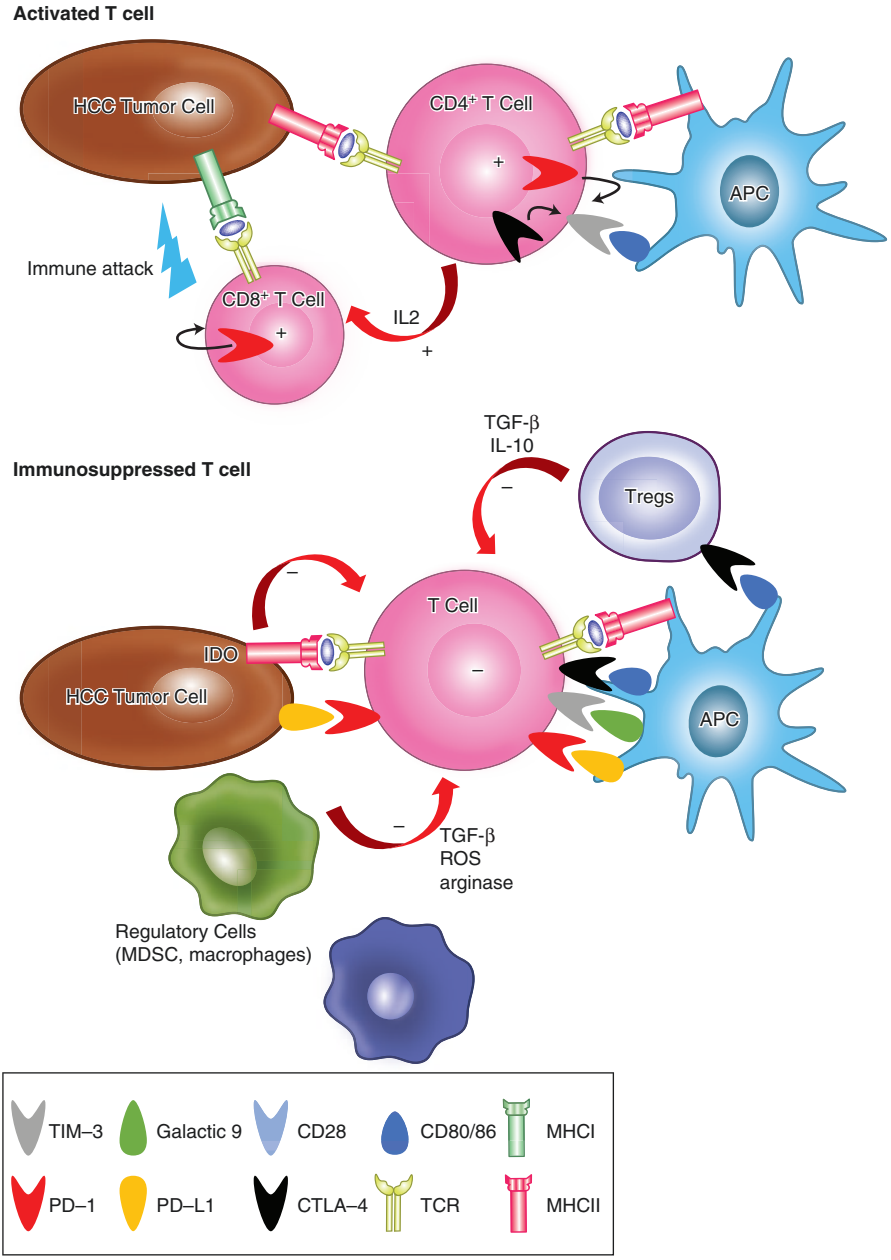

Fig. 12.1 Overview of tumor immune response. Under physiological conditions, antigens are recognized and presented to CD4+ T cells, which further activate CD8+ T cells. T cell activation causes upregulation of CTLA-4 and PD-1 to prevent overactivation of the immune response. The tumor microenvironment creates an immunosuppressive milieu through recruitment of regulatory T cells (Tres), myeloid-derived suppressor cells (MDSCs), and upregulation of immune checkpoints. These immunosuppressive factors lead toward carcinogenesis and tumor progression

In addition to the aforementioned checkpoint inhibitors, other inhibitory membrane proteins are being recognized as creating an immunosuppressive TME and therefore may represent a potential therapeutic target. TIM-3 is expressed by cells of the innate and adaptive immune system. Galactin-9 is a ligand for TIM-3 and is expressed by multiple tissues, including the liver, and regulates cell differentiation, adhesion, and cell death. Galactin-9 and TIM-3 have been shown to inhibit the T cell response. In addition, TIM-3-expressing T cells often co-express PD-1, indicating the two inhibitory pathways may cooperate to produce a severely exhausted T cell phenotype [9]. Preclinical studies have shown that blocking TIM-3 and PD-1 may produce a greater tumor response in non-small cell lung cancer models [17].

LAG-3 has also been shown to act synergistically with PD-1 to promote tumor immune evasion. LAG-3 is expressed by activated T cells and binds to MHC class II molecules on DCs, thereby decreasing the costimulatory function of DCs and is a hallmark of exhausted T cells [1, 9]. Other immune-inhibitory factors include arginase-1 which is expressed at high levels by MDSCs and tumor-associated machrophages (TAMs) as well as indoleamine 2,3-dioxygenase (IDO) which is expressed by multiple cell types in the TME [1]. Both arginase-1 and IDO metabolize amine acids, arginine and tryptophan, respectively, which deprive immune cells of vital nutrients and generate immunosuppressive by-products [1].

Immunotherapies

As we discussed, the tumor microenvironment creates an immunosuppressive milieu promoting tumor formation and limits the capacity of the host to mount a proper immune response. Investigations are ongoing to create immune-based therapies to promote tumor recognition and ultimately tumor eradication. Here, we discuss various approaches to immune-based therapies, including the application of immune checkpoint inhibitors, cell-based therapies, and cytokine-based therapies. We will then discuss future directions with genetically engineered therapies including chimeric antigen receptors (CAR) T cell therapies and TCR therapies.

Immune Checkpoint Inhibitors

Immune checkpoint inhibitors have gained interest for patients with advanced HCC after success in treating patients with melanoma and non-small cell lung cancer with these agents. Between 2013 and 2017, results of three clinical trials have been reported for patients with advanced HCC treated with immune checkpoint inhibitors; two trials using tremelimumab (anti-CTLA-4), and one trial utilizing nivolumab (anti-PD1) (Table 12.1). Checkpoint inhibitors have several advantages over other types of immunotherapies such as cell-based therapies with regard to commercial availability, wider applicability to a range of pathologic conditions, and no restriction by HLA status.

Table 12.1 Clinical trial results utilizing immune checkpoint inhibitors in HCC

	Sangro et al. [18]	Duffy et al. [19]	El-Khoueiry et al. [4]
Agent (target)	Tremelimumab (CTLA-4)	Tremelimumab (CTLA-4)	Nivolumab (PD-1)
Number of patients evaluated	17	19	212
Complete response (CR)	0	0	3 (1%)
Partial response (PR)	3 (18%)	5 (26%)	39 (18%)
Stable disease (SD)	10 (59%)	12 (63%)	96 (45%)
Median time to progression	6.5 months	7.4 months	4.1 months

Sangro et al. reported the first clinical use of immune checkpoint inhibitor, tremelimumab, in a phase II trial in patients with chronic hepatitis C and advanced HCC not amenable to surgical or locoregional therapies [18]. Fifty-seven percent of patients enrolled progressed on previous therapies, and 43% had severer liver disease, i.e., Child-Pugh class B advanced fibrosis/cirrhosis. This is significant given that previously evaluated drugs such as sorafenib have been tested in mostly patients with Child-Pugh class A disease and better natural history. Although the patients received what is now considered to be a suboptimal dose of tremelimumab (15 mg/kg every 90 days to a maximum of four doses), partial response (PR) in 3 patients and stable disease (SD) in 10 patients were observed among 17 evaluable patients [9]. In addition, tremelimumab was generally well tolerated, and a reduction in HCV viral load was also detected.

The next trial used tremelimumab in combination with noncurative tumor ablation utilizing radiofrequency ablation (RFA) or transarterial chemoembolization (TACE) [19]. The hypothesis of adding tumor ablation was the assumption that RFA or TACE may cause immunogenic cell death. This type of cell death could then lead to a systemic release of antigens and a global immune response which may be enhanced by the checkpoint inhibitor [9, 19]. This was a phase I/II study with optimal therapeutic dosing of tremelimumab consisting of 78% of patients who advanced on previous therapies. Fourteen percent of patients were Child-Pugh class B and 75% had viral hepatitis. Of the 19 evaluable patients, 5 patients (26%) had a PR and 12 patients (63%) were deemed to have SD. Again, this trial showed an acceptable safety profile, reduction in viral load, and promising antitumor effects.

The encouraging results of the tremelimumab studies followed into utilizing nivolumab in CheckMate 040 trial [4]. This study consisted of dose-escalation and dose-expansion phases in patients with intermediate or advanced HCC who had progressed, were intolerant, or refused sorafenib. As a result of the dose-escalation study, 3 mg/kg every 2 weeks was chosen for the expansion cohort. Most patients had advanced HCC with extrahepatic metastases and received sorafenib. Of the 212 evaluable patients, they observed complete response (CR) in 3 patients (1%) and PR in 39 patients (18%). Furthermore, response rates were similar in both patients with or without prior sorafenib therapy. These findings resulted in nivolumab being

approved in the second-line setting for patients with advanced HCC. Another anti-PD1, pembrolizumab, has shown similar objective response rate of 17% in 104 enrolled patients in a phase II trial (KEYNOTE-224) [20]. A follow-up phase III study however did not meet the primary end points for OS and PFS despite favorable trends, suggesting the need for predictive biomarkers of response (KEYNOTE-240, NCT02702401). Another phase III trial of pembrolizumab is ongoing in Asian patients (KEYNOTE-394, NCT03062358). A phase II trial is underway to evaluate the agent as neoadjuvant therapy following surgical resection or ablation (AURORA, NCT03337841).

Although these aforementioned trials showed promising results for the use of immune checkpoint inhibitors in patients with advanced HCC, additional trails are needed and underway to show efficacy as first-line therapy and in combination with other immune or cytotoxic therapies. In patients with melanoma combining nivolumab and ipilimumab (anti-CTLA-4) produced a greater objective-response rate and progression-free survival as compared to single-agent therapy [21]. Currently, phase III clinical trials are underway with nivolumab in the first-line setting against sorafenib (CheckMate 459) and combination therapies of tremelimumab and durvalumab (anti-PD-L1) along with ablative therapies (NCT02821754) and nivolumab plus sorafenib (NCT03439891).

Cell Bases Therapies

Adoptive cell transfer (ACT) is a highly personalized form of cancer immunotherapy that involves the transfer of host-derived expanded immune cells [22]. Adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) has been shown to produce a complete and durable tumor regression in patients with metastatic melanoma [23]. There is limited data in the treatment of HCC patients with metastatic or unresectable disease via the transfer of ex vivo expanded autologous TIL. However, ACT has been tried in the adjuvant setting. Takayama et al. studied ACT in the adjuvant setting in 150 patients who had undergone curative resection for HCC with 76 patients receiving adoptive immunotherapy and 74 patients receiving no adjuvant treatment [24]. The ACT treatment consisted of adjuvant activated autologous lymphocyte infusions. ACT treatment increased the recurrence-free survival (RFS) but had no impact on overall survival (OS). Activated T cell transfer has also been applied with adjunctive treatments such as an autologous tumor lysate-pulsed DC vaccine [25]. In this study, patients who underwent a curative HCC liver resection received an adjuvant DC vaccine which was made from a patient's isolated DCs pulsing with tumor lysate created from the resected tumor along with $CD3^+$ activated T cells. There was a significant difference in both RFS and OS in favor of combination DC vaccine and ACT vs. no adjuvant therapy. The difference between these two studies may highlight the need for combination therapy strategies in the future.

Another strategy of ACT that has been tried in the adjuvant setting for HCC is through the use of cytokine-induced killer (CIK) cells. CIK cells are autologous cells that are expanded ex vivo from a patient's peripheral blood mononuclear cells (PBMCs) which are cultured with a cytokine cocktail and anti-CD3 antibodies. CIK cells consist of a variety of subpopulations: $CD3^+/CD56^+$ cells, $CD3^-/CD56^+$ NK cells, and $CD3^+/CD56^-$ cytotoxic T cells. Therefore, CIK cells have potent antitumor effects with the dual-functional capability of T cells and NK cells. The result indicated its substantial specificity toward tumor cells and the capability of acting independent of TCR [26, 27]. Lee et al. studied the use of CIK cells in the adjuvant setting in patients with resected HCC. The primary end point of this study is RFS and secondary end points include OS and cancer-specific survival. They found that CIK cell immunotherapy was associated with improved RFS, OS, and cancer-specific survival [27].

CIK cell therapy has also been evaluated in the setting of inoperable advanced disease. In a nonrandomized evaluation of patients receiving RFA and TACE, CIK cell therapy was shown to possibly improve OS when given with these locoregional therapies [28]. Additionally, in a phase II randomized trial, it was found that CIK cell therapy can improve OS and PFS as compared to standard treatment [29]. Additional data support the use of tadalafil, a phosphodiesterase type 5 (PDE5) inhibitor, in combination with CIK cell therapy, as it improves efficacy through the suppression of MDSC activity (unpublished work from Greten lab). These studies demonstrate promise in adoptive cell-transfer techniques for patients with advanced nonoperable HCC as well as in the adjuvant setting. However more research and clinical trials are needed in the application of these treatment methods. A major potential drawback of ACT is the need of specialized centers and the difficultly in making these treatments widely and commercially available.

Cytokines

Dr. Steven Rosenberg pioneered one of the first successful immune-based treatments with the application of interleukin-2 (IL-2) for the treatment of metastatic melanoma [30]. However, for patients with HCC, cytokines-based treatments have met with limited success. The use of interferon (IFN) appeared as a logical first choice for the treatment of HCC, and it may show both antiviral and antitumor functions. However, for patients with advanced disease, the tumor response rates to IFN therapy was poor with a partial response rate of 6% (2 of 30 patients) and no benefit in OS [31]. Additionally, IFN therapy was not well tolerated in patients with cirrhosis and HCC resulting in nearly half of the patients discontinuing treatment due to intolerance or adverse events [31]. IFN has also been studied in the adjuvant setting for patients with viral hepatitis-related HCC with conflicting results. Chen et al. investigated the use of adjuvant IFN and failed to find a statistical difference in either RFS or OS [32]. Sun et al. investigated a similar cohort with IFN and found no difference in disease-free survival but found a significant difference in OS in

favor of patients receiving adjuvant IFN [33]. Of note, more patients in the IFN-group received a second liver resection than patients in the control group. Although this was not statistically significant, this may influence OS in these patients.

Additional trials with cytokines involve phase I trials with intratumoral delivery of IL-12 [34, 35]. Both these trials included patients with advanced GI tumors, displaying feasibility and safety of the therapy, but did not show promising antitumor response rates, although the studies were underpowered. Further investigations are underway with promising results involving a small molecule inhibitor of TGF-ß receptor 1, LY2157299 (galunisertib) in patients with advanced HCC either alone or in combination with nivolumab or stereotactic body radiotherapy [10].

Vaccine Therapy

The introduction of the hepatitis B vaccine in the 1980s may have virtually restructured the landscape of HCC by preventing assumedly numerous cases of HCC. Similarly, immunization against human papilloma virus has greatly decreased the risk of cervical cancer in a similar fashion. Utilizing similar principles of immune recognition and promoting an adaptive immune response against specific antigens, i.e., vaccination, can be applied not only for cancer prevention but also for cancer treatment.

The basic element underlying cancer vaccination is increased immune recognition of tumor-specific neoantigens that result from either driver or passenger genomic DNA mutations, producing altered proteins to create neoepitopes. Multineoepitope vaccines have been shown to activate both neoantigen-specific $CD4^+$ and $CD8^+$ T cells. In the first priming phase of the immune response, cross presentation between DCs and T_H1 cells can induce potent neoepitope-specific cytotoxic T lymphocytes (CTLs) with improved tumor penetration and generating memory $CD8^+$ T cells. In the tumor, the vaccine-induced T_H1 $CD4^+$ T cells can further promote an inflammatory TME through increased IFN-γ, thus upregulating MHC class I molecules on tumor cells, which improves killing by the $CD8^+$ T cells. Additionally, the IFN-γ upregulates MHC class II molecules, further sensitizing tumors to recognition by $CD4^+$ T cells [36].

AFP is expressed by a fraction of HCC tumors and during the process of fetal development but not in healthy adult tissues. AFP was the first TAA targeted for vaccine-based therapies in HCC despite limited success. In early studies utilizing AFP peptides or AFP-pulsed DCs, a T cell response was induced, but no clinical benefit of the therapy was observed [37, 38]. In a more recent study, administration of AFP-derived peptides to 15 HCC patients produced T cells that reacted to the peptides and resulted in CR in 1 patient and SD in 8 patients with no adverse events [39].

Other trials have been conducted utilizing peptide vaccine against a carcinoembryonic antigen, GPC3, which is an appealing target for HCC vaccines because of its specificity to HCC and association with a poor prognosis of the patients. Early studies utilizing a GPC3 peptide vaccine found the treatment to be safe and able to

induce tumor infiltration of $CD8^+$ T cells. However, the therapy produced only 1 PR out of 33 treated patients with a median time to tumor progression of 3.4 months [40]. Preclinical studies have shown that utilizing anti-PD1 therapy may result in increased response to GPC3 peptide vaccines [41]. The GPC3 vaccine was also tested in the adjuvant setting, demonstrating a significantly improved recurrence rate in patients treated with surgery plus vaccine compared to surgery alone at 1 year but was found to be no longer statistically significant at 2 years [42]. Besides a vaccine, targeting GPC3 through an anti-GPC3 antibody, GC33, has shown to be tolerated and may have promise in further phase II trials [43].

Clinical trials have also been performed using the targeted oncolytic poxvirus, JX-594, which was designed to replicate in and destroy cancer cells. JX-594 was found to be safe in a phase I study and displayed some promising results in a phase II trial with a intrahepatic disease control rate of 46% [44, 45]. Currently, there is an ongoing phase III clinical trial evaluating JX-594 (Pexa Vec) followed by sorafenib versus sorafenib alone in patients with advanced HCC (NCT02562755). Other trials utilizing a vaccine-based strategy with DCs pulsed with antigens have failed to demonstrate a significant clinical benefit [10]. Additionally, a phase II trial of low-dose cyclophosphamide in combination with the telomerase peptide GV1001 in patients with advanced HCC showed no radiologically detectable tumor responses [46]. The above studies demonstrate that although vaccine-based strategies in HCC can mount an immune response as shown by antigen-specific T cells in the blood of treated patients, local factors in the tumor likely prevent tumor eradication. Therefore, further studies are needed to progress vaccine-based immunotherapies, perhaps in combination with other immune therapies, in patients with advanced HCC.

Sensitivity and Resistance to Immune Therapies

Although there has been recent success with immunotherapy in HCC, namely, immune checkpoint inhibitors, a subset of patients do not respond to therapy. Unfortunately, there is little known regarding the characteristics of HCC tumors that may predict response to immunotherapies. Experimental evidence suggests high levels of TIL, IFN signaling, presence of immune checkpoints, or a high tumor mutation burden may favor a positive clinical response to immune-based therapies. Furthermore, gene expression profiles of tumor, stromal, and immune cells indicate approximately 25% of evaluated HCC samples express markers of an inflammatory response which may indicate better sensitivity to immunotherapy [47].

Recent studies have suggested potential mechanisms underlying resistance to and escape from immunotherapies. Resistance to immunotherapy can be classified as primary resistance or adaptive and acquired resistance. Primary resistance occurs when the tumor does not respond to an immunotherapy from the initiation of therapy likely through lack of tumor antigen recognition by T cells. Adaptive or acquired resistance may occur when a tumor is recognized by the immune system, but the

tumor protects itself from immune attack. Additionally, resistance can be due to intrinsic tumor properties such as a low mutation burden and high PD-L1 expression or extrinsic tumor properties such as a highly immunosuppressive TME and lack of T cells with antigen-specific TCRs [48].

Further proposed mechanisms of resistance to immunotherapy include tumor cells, which have lost beta$_2$-microglobulin (ß$_2$-m) in tumor cells and can therefore no longer be recognized by CD8$^+$ T cells [49]. Others observed that nonresponders to anti-CLTA-4 with metastatic melanoma have tumors with genomic defects in IFN-γ pathway genes [50]. Additionally, upregulation of alternative immune checkpoints, notably TIM-3, have also been found in lung adenocarcinoma. This suggests the upregulation of alternative immune checkpoints may be associated with adaptive resistance to anti-PD-1 therapy and therefore TIM-3 may be a potential target for combination therapy with anti-PD-1 [17]. Further understanding of the underlying resistance mechanisms to immunotherapies is needed to develop proper combination strategies to improve efficacy of checkpoint blockade for HCC treatment.

Future Therapies

Genetically modified T cells have been applied to cancer therapy, and this technique is likely to be applied to HCC in the near future. T cells can be manipulated to express a high-affinity TCR or a chimeric antigen receptor (CAR). TCRs have a limitation of being restricted to recognize specific MHC molecules and therefore can only be used in patients who possess those specific HLAs. CAR T cells express a genetically engineered fusion molecule that act independently from an MHC molecule and are therefore able to circumvent HLA restrictions. The use of CAR T cells has been thrust into mainstream treatment for patients with CD19$^+$ hematologic malignancies. The use of TCR and CAR T cells for patients with HCC remains in its infancy. Preclinical studies utilizing GPC3-specific T cells showed promising results in HCC-bearing mice [51]. A phase I study is currently underway to investigate its safety and antitumor activity of autologous T cells expressing TCRs for AFP in patients with advanced HCC (NCT03132792).

As alluded to earlier in this chapter, combining multiple immunotherapy modalities may improve HCC response rates. The combination of checkpoint inhibitors, ACT, cytokines, and vaccines have been studied in other malignancies with varying degrees of success. The application of these approaches is likely soon to be applied to HCC. The use of immune-based therapies also raises concern about safety of the agents in HCC patients with underlying liver dysfunction caused by viral hepatitis or other etiologies. An early concern was the possible hepatocyte damage due to an overwhelming immune response against viruses in infected hepatocytes. However, the earlier application of immunotherapy, namely, checkpoint inhibitors, appeared to be generally safe and well-tolerated in patients with advanced HCC and therefore has led to the pursuit of combination with other treatment modalities [52].

Additionally, effective adjuvant therapy is lacking in HCC, and the use of immunotherapies may provide a benefit to this patient population and requires further evaluation. Phase I and II clinical trials are planned to test nivolumab as neoadjuvant therapy after surgical resection of HCC tumors (NCT03510871, NCT03299946).

Conclusion

The application of immunotherapies has restructured the treatment approach to numerous malignancies such as melanoma and non-small cell lung cancer and is now being adopted for the treatment of HCC patients. Although the liver microenvironment is immunosuppressive and chronic liver disease contributes to further immune tolerance, the early application of immune-based therapies has demonstrated promising results in clinical trials. Further basic, translational, and clinical studies are required to better understand the complex interactions between tumor cells, immune cells, and immunotherapies in the tumor microenvironment to develop a treatment strategy to eliminate HCC cells.

References

1. Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2015;12(12):681–700. https://doi.org/10.1038/nrgastro.2015.173.
2. Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology. 1998;27(2):407–14. https://doi.org/10.1002/hep.510270214.
3. Greten TF, Duffy AG, Korangy F. Hepatocellular carcinoma from an immunologic perspective. Clin Cancer Res. 2013;19(24):6678–85. https://doi.org/10.1158/1078-0432.ccr-13-1721.
4. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017;389(10088):2492–502. https://doi.org/10.1016/s0140-6736(17)31046-2.
5. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58. https://doi.org/10.1126/science.1235122.
6. Yarchoan M, Johnson BA 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer. 2017;17(4):209–22. https://doi.org/10.1038/nrc.2016.154.
7. Flecken T, Schmidt N, Hild S, Gostick E, Drognitz O, Zeiser R, et al. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. Hepatology. 2014;59(4):1415–26. https://doi.org/10.1002/hep.26731.
8. Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y, Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2015;21(37):10573–83. https://doi.org/10.3748/wjg.v21.i37.10573.
9. Greten TF, Sangro B. Targets for immunotherapy of liver cancer. J Hepatol. 2017; https://doi.org/10.1016/j.jhep.2017.09.007.

10. Makarova-Rusher OV, Medina-Echeverz J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. J Hepatol. 2015;62(6):1420–9. https://doi.org/10.1016/j.jhep.2015.02.038.
11. Inarrairaegui M, Melero I, Sangro B. Immunotherapy of hepatocellular carcinoma: facts and hopes. Clin Cancer Res. 2018;24(7):1518–24. https://doi.org/10.1158/1078-0432.Ccr-17-0289.
12. Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res. 2009;15(3):971–9. https://doi.org/10.1158/1078-0432.Ccr-08-1608.
13. Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. Cancer Res. 2005;65(6):2457–64. https://doi.org/10.1158/0008-5472.Can-04-3232.
14. Greten TF, Wang XW, Korangy F. Current concepts of immune based treatments for patients with HCC: from basic science to novel treatment approaches. Gut. 2015;64(5):842–8. https://doi.org/10.1136/gutjnl-2014-307990.
15. Arihara F, Mizukoshi E, Kitahara M, Takata Y, Arai K, Yamashita T, et al. Increase in CD14+HLA-DR −/low myeloid-derived suppressor cells in hepatocellular carcinoma patients and its impact on prognosis. Cancer Immunol Immunother. 2013;62(8):1421–30. https://doi.org/10.1007/s00262-013-1447-1.
16. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Kruger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. Gastroenterology. 2008;135(1):234–43. https://doi.org/10.1053/j.gastro.2008.03.020.
17. Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun. 2016;7:10501. https://doi.org/10.1038/ncomms10501.
18. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, Barrera P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. J Hepatol. 2013;59(1):81–8. https://doi.org/10.1016/j.jhep.2013.02.022.
19. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. J Hepatol. 2017;66(3):545–51. https://doi.org/10.1016/j.jhep.2016.10.029.
20. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018;19(7):940–52. https://doi.org/10.1016/S1470-2045(18)30351-6.
21. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372(21):2006–17. https://doi.org/10.1056/NEJMoa1414428.
22. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348(6230):62–8. https://doi.org/10.1126/science.aaa4967.
23. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17(13):4550–7. https://doi.org/10.1158/1078-0432.Ccr-11-0116.
24. Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet. 2000;356(9232):802–7. https://doi.org/10.1016/S0140-6736(00)02654-4.
25. Shimizu K, Kotera Y, Aruga A, Takeshita N, Katagiri S, Ariizumi S, et al. Postoperative dendritic cell vaccine plus activated T-cell transfer improves the survival of patients with invasive hepatocellular carcinoma. Hum Vaccin Immunother. 2014;10(4):970–6.

26. Schmidt-Wolf IG, Lefterova P, Mehta BA, Fernandez LP, Huhn D, Blume KG, et al. Phenotypic characterization and identification of effector cells involved in tumor cell recognition of cytokine-induced killer cells. Exp Hematol. 1993;21(13):1673–9.
27. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, Yu SJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology. 2015;148(7):1383–91 e6. https://doi.org/10.1053/j.gastro.2015.02.055.
28. Huang ZM, Li W, Li S, Gao F, Zhou QM, Wu FM, et al. Cytokine-induced killer cells in combination with transcatheter arterial chemoembolization and radiofrequency ablation for hepatocellular carcinoma patients. J Immunother (Hagerstown, MD: 1997). 2013;36(5):287–93. https://doi.org/10.1097/CJI.0b013e3182948452.
29. Yu X, Zhao H, Liu L, Cao S, Ren B, Zhang N, et al. A randomized phase II study of autologous cytokine-induced killer cells in treatment of hepatocellular carcinoma. J Clin Immunol. 2014;34(2):194–203. https://doi.org/10.1007/s10875-013-9976-0.
30. Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol. 2014;192(12):5451–8. https://doi.org/10.4049/jimmunol.1490019.
31. Llovet JM, Sala M, Castells L, Suarez Y, Vilana R, Bianchi L, et al. Randomized controlled trial of interferon treatment for advanced hepatocellular carcinoma. Hepatology. 2000;31(1):54–8. https://doi.org/10.1002/hep.510310111.
32. Chen LT, Chen MF, Li LA, Lee PH, Jeng LB, Lin DY, et al. Long-term results of a randomized, observation-controlled, phase III trial of adjuvant interferon Alfa-2b in hepatocellular carcinoma after curative resection. Ann Surg. 2012;255(1):8–17. https://doi.org/10.1097/SLA.0b013e3182363ff9.
33. Sun HC, Tang ZY, Wang L, Qin LX, Ma ZC, Ye QH, et al. Postoperative interferon alpha treatment postponed recurrence and improved overall survival in patients after curative resection of HBV-related hepatocellular carcinoma: a randomized clinical trial. J Cancer Res Clin Oncol. 2006;132(7):458–65. https://doi.org/10.1007/s00432-006-0091-y.
34. Mazzolini G, Alfaro C, Sangro B, Feijoo E, Ruiz J, Benito A, et al. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. J Clin Oncol. 2005;23(5):999–1010. https://doi.org/10.1200/jco.2005.00.463.
35. Sangro B, Mazzolini G, Ruiz J, Herraiz M, Quiroga J, Herrero I, et al. Phase I trial of intratumoral injection of an adenovirus encoding interleukin-12 for advanced digestive tumors. J Clin Oncol. 2004;22(8):1389–97. https://doi.org/10.1200/jco.2004.04.059.
36. Sahin U, Tureci O. Personalized vaccines for cancer immunotherapy. Science. 2018;359(6382):1355–60. https://doi.org/10.1126/science.aar7112.
37. Butterfield LH, Economou JS, Gamblin TC, Geller DA. Alpha fetoprotein DNA prime and adenovirus boost immunization of two hepatocellular cancer patients. J Transl Med. 2014;12:86. https://doi.org/10.1186/1479-5876-12-86.
38. Butterfield LH, Ribas A, Meng WS, Dissette VB, Amarnani S, Vu HT, et al. T-cell responses to HLA-A*0201 immunodominant peptides derived from alpha-fetoprotein in patients with hepatocellular cancer. Clin Cancer Res. 2003;9(16. Pt 1):5902–8.
39. Nakagawa H, Mizukoshi E, Kobayashi E, Tamai T, Hamana H, Ozawa T, et al. Association between high-avidity T-cell receptors, induced by alpha-fetoprotein-derived peptides, and anti-tumor effects in patients with hepatocellular carcinoma. Gastroenterology. 2017;152(6):1395–406.e10. https://doi.org/10.1053/j.gastro.2017.02.001.
40. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. Clin Cancer Res. 2012;18(13):3686–96. https://doi.org/10.1158/1078-0432.Ccr-11-3044.
41. Sawada Y, Yoshikawa T, Shimomura M, Iwama T, Endo I, Nakatsura T. Programmed death-1 blockade enhances the antitumor effects of peptide vaccine-induced peptide-specific cytotoxic T lymphocytes. Int J Oncol. 2015;46(1):28–36. https://doi.org/10.3892/ijo.2014.2737.

42. Sawada Y, Yoshikawa T, Ofuji K, Yoshimura M, Tsuchiya N, Takahashi M, et al. Phase II study of the GPC3-derived peptide vaccine as an adjuvant therapy for hepatocellular carcinoma patients. Oncoimmunology. 2016;5(5):e1129483. https://doi.org/10.1080/2162402x.2015.1129483.
43. Zhu AX, Gold PJ, El-Khoueiry AB, Abrams TA, Morikawa H, Ohishi N, et al. First-in-man phase I study of GC33, a novel recombinant humanized antibody against glypican-3, in patients with advanced hepatocellular carcinoma. Clin Cancer Res. 2013;19(4):920–8. https://doi.org/10.1158/1078-0432.CCR-12-2616.
44. Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, Bloomston M, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat Med. 2013;19(3):329–36. https://doi.org/10.1038/nm.3089.
45. Park BH, Hwang T, Liu TC, Sze DY, Kim JS, Kwon HC, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. Lancet Oncol. 2008;9(6):533–42. https://doi.org/10.1016/s1470-2045(08)70107-4.
46. Greten TF, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, et al. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. BMC Cancer. 2010;10:209. https://doi.org/10.1186/1471-2407-10-209.
47. Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. Gastroenterology. 2017;153(3):812–26. https://doi.org/10.1053/j.gastro.2017.06.007.
48. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707–23. https://doi.org/10.1016/j.cell.2017.01.017.
49. Restifo NP, Marincola FM, Kawakami Y, Taubenberger J, Yannelli JR, Rosenberg SA. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. J Natl Cancer Inst. 1996;88(2):100–8.
50. Gao J, Shi LZ, Zhao H, Chen J, Xiong L, He Q, et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell. 2016;167(2):397–404. e9. https://doi.org/10.1016/j.cell.2016.08.069.
51. Gao H, Li K, Tu H, Pan X, Jiang H, Shi B, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. Clin Cancer Res. 2014;20(24):6418–28. https://doi.org/10.1158/1078-0432.Ccr-14-1170.
52. Brown ZJ, Heinrich B, Steinberg SM, Yu SJ, Greten TF. Safety in treatment of hepatocellular carcinoma with immune checkpoint inhibitors as compared to melanoma and non-small cell lung cancer. J Immunother Cancer. 2017;5(1):93. https://doi.org/10.1186/s40425-017-0298-2.

Chapter 13
Prevention Strategies for Hepatocellular Carcinoma

Derek J. Erstad, Allen A. Razavi, Shen Li, Kenneth K. Tanabe, and Bryan C. Fuchs

Rationale for HCC Prevention

Hepatocellular carcinoma (HCC) is the fifth most common cancer with rising global incidence [1]. Surgical resection, liver transplant, and ablation provide the only potential curative therapies, though over 70% of patients are diagnosed at advanced stages of disease. Unfortunately, medical therapies have been largely ineffective, and HCC mortality remains high, with a 5-year survival of less than 15% in the United States [2]. Moreover, despite rigorous evaluation of screening strategies, surveillance noncompliance remains a limiting factor for early diagnosis and curative treatment [3–5]. HCC arises from a milieu of chronic inflammation and persistent liver injury that progresses over decades and occurs 40 times more often among cirrhotics [6, 7]. Therefore, there is potential for identification of at-risk individuals and development of preventative strategies. For these reasons, prevention is an attractive alternative approach for HCC management.

There are three main approaches to HCC prevention, primary, secondary, and tertiary, which are distinguished by a previous history of chronic liver disease and HCC [8]. The purpose of primary HCC prevention is to identify and abolish risk factors for chronic liver disease, and among patients with already established cirrhosis, to treat underlying chronic liver conditions prior to the onset of HCC [9]. Multiple primary and secondary preventative strategies have been proposed, though few have been validated in prospective, randomized controlled trials (RCTs). Particular strategies have increased relevance based on geography and context. Endemic HBV in

D. J. Erstad · S. Li · K. K. Tanabe · B. C. Fuchs (✉)
Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: BFUCHS@mgh.harvard.edu

A. A. Razavi
Tufts University School of Medicine, Boston, MA, USA

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_13

East Asia and Sub-Saharan Africa presents a major source of cirrhosis and HCC, and therefore, implementation of vaccination programs stand to reduce the burden of hepatitis-mediated HCC in these regions [10]. Similarly, aflatoxin exposure remains a public health issue in East Asia, and policy changes to reduce dietary toxin exposure is another potential high-yield intervention [11]. In contrast, in Western nations, obesity-related nonalcoholic steatohepatitis (NASH) is the fastest rising cause of liver failure and HCC [12, 13]. In this regard, more focus has been placed on bariatric surgery and anti-inflammatory pharmacologic agents that are used to treat metabolic syndrome-related disorders, including aspirin, statins, cyclooxygenase inhibitors, and anti-diabetic agents [14]. There is also great interest in dietary supplements, notably coffee and green tea polyphenols, both of which are thought to have anti-inflammatory and anticancer effects. Hepatitis C virus (HCV) is highly prevalent on a global scale, and the recent development of effective direct acting antivirals (DAAs) offers great potential not only for curative therapy in patients with chronic viral hepatitis but secondarily to reduce the transmission and incidence of HCV [15].

Tertiary prevention strategies are designed to reduce the risk of HCC recurrence after curative resection of primary disease, typically in patients with baseline chronic liver disease or cirrhosis. Many of the same dietary and pharmacologic agents that are under investigation for secondary prevention are also being studied in the tertiary context. In addition, adjuvant chemotherapeutic agents targeting cancer are also under investigation. In this chapter, we systematically review HCC prevention strategies, their indications and limitations, and future directions for HCC prevention.

Primary and Secondary Prevention Strategies

HCC is a malignancy borne from chronic hepatic insults, and approximately 90% of cases occur in the setting of cirrhosis. The annual incidence of HCC among cirrhotics is approximately 2–5% [16, 17]. The most common etiologies for chronic liver disease and cirrhosis include viral infection, alcohol consumption, obesity and metabolic syndromes, autoimmune and genetic disorders, and toxin exposure [18]. Despite each having unique molecular mechanisms, these processes result in a common pathogenic pathway characterized by persistent hepatocyte injury, chronic inflammation, progressive hepatic fibrosis, and ultimately hepatocarcinogenesis and HCC development [19]. In the sections below, we review these common etiologies of chronic liver disease and targeted efforts for HCC prevention.

Viral Hepatitis

Approximately 80% of new HCC cases occur in developing countries, of which 80% are attributable to HCV and HBV infection [20]. In the United States and Europe, untreated HCV infection accounts for 25–75% of HCC cases [21]. Among

HCV and HBV carriers, the risk of HCC is increased by approximately 17-fold and 15-fold, respectively [22]. Viral oncogenesis is a complex multistep process that initiates with hepatocyte infection, injury, and subsequent inflammation. Autocrine and paracrine signaling feedback loops between hepatocytes and stellate cells establish a pro-survival, chronically inflamed microenvironment with reduced immune surveillance that is optimal for hepatocyte transformation [23]. HCV and HBV have unique molecular mechanisms that contribute to this process, as discussed below.

HCV Epidemiology and Pathogenesis

Approximately 3% of the world's population is infected with HCV, which is a single-strand RNA virus from the *Flaviviridae* family. The annual HCC incidence among patients with cirrhosis and active HCV infection ranges from 1% to 8%; however, with effective treatment, evidenced by sustained viral response (SVR), this incidence decreases to 1% or less [24, 25]. Unlike HBV, the risk of HCV-related HCC increases with the degree of cirrhosis, and HCC is rarely observed in the absence of hepatic fibrosis [26]. There is conflicting evidence regarding the association between serum viral RNA load and HCC risk [27, 28]. Due to a high viral mutation rate, attempts at vaccine development against HCV have been unsuccessful but remain an active area of investigation even after the successful development of effective direct acting antivirals (DAAs).

HCV often presents with a mild acute phase and the majority of patients are unaware of their infection status until signs of chronic liver disease present several decades later. In the United States, approximately 1.6% of the populace is infected with HCV; however, less than 25% of individuals are aware of their infection status, and this rate decreases to less than 5% globally [29]. In the United States, the majority of HCV infections occurred among the "baby boomer" generation (prevalence of nearly 3%), and therefore the Centers for Disease Control (CDC) recommends HCV testing for anyone born between the years 1945 and 1965 [30]. Approximately 80% of HCV infections progress to chronic hepatitis, of which 15% will progress to cirrhosis [31]. It is projected that without treatment, 14% of HCV patients will eventually develop HCC [32]. Given the chronic nature of HCV, there is theoretically ample opportunity for preventive intervention. However, because the disease is frequently silent for many years and regular HCV testing is not utilized in most countries, there is still the hurdle of identifying patients who are infected.

The mechanism of HCV-induced hepatocarcinogenesis is not well established, though the oncogenic effects are likely secondary to viral proteins as the virus is unable to stably integrate into the genome. HCV core protein is capable of inhibiting multiple tumor suppressors including p21, p53, and Rb, and has also been associated with CDH1 downregulation via promoter hypermethylation, TGF-β upregulation, and activation of MEK/ERK phosphosignaling [33–35]. On a physiologic level, HCV core protein has been shown to influence multiple cellular processes, including proliferation, survival, lipid metabolism, reactive oxygen species (ROS) generation, and immune interactions [36]. In particular, HCV core protein, as well as another

HCV protein, NS5A, has been shown to impair β-oxidation, resulting in reduced mitochondrial electron transport chain function, endoplasmic reticulum (ER) stress, impaired lipid metabolism leading to hepatic steatosis, and increased ROS formation [37, 38]. Given the interdependence of these metabolic functions, dysregulation of any given process can further deregulate cellular homeostasis in a feed-forward manner. Over time, HCV-induced hepatocyte dysfunction and cell death establishes an inflammatory microenvironment that drives stellate-cell activation, fibrogenesis, and altered immune surveillance, which are conditions that have been shown to support hepatocyte transformation.

HCV-Targeted Prevention

Interferon (IFN)-based therapy was introduced in 1986 and served as the mainstay of HCV treatment prior to the development of DAAs. IFN activates the immune system, which frequently results in enhanced viral detection and clearance. SVR rates of 50–70% in genotype 1/4 HCV patients without advanced fibrosis were achievable using pegylated interferon (PegIFN) in combination with ribavirin, a nucleoside inhibitor [39]. However, among patients with bridging fibrosis or cirrhosis, SVR was observed in only 51% and 33%, respectively. Given the immune-stimulating effects of IFN, treatment is often associated with severe adverse effects. Thus, low efficacy and high toxicity limit the utility of IFN-based HCV treatment.

Advances in our understanding of molecular virology led to the development of DAAs, which were introduced in 2011, creating a paradigmatic shift in our ability to safely and effectively treat HCV. Initially, first-generation NS3-4A protease inhibitors, telaprevir and boceprevir, in combination with PegIFN and ribavirin were shown to achieve SVR rates of 65–75%, resulting in their Food and Drug Administration (FDA) approval for HCV genotype 1 treatment [40, 41]. This was followed by approval of Simeprevir, another NS3-4A inhibitor, in 2013 [42]. However, development of NS5B polymerase inhibitors led to a critical breakthrough in achieving SVR. Sofosbuvir, a nucleotide analogue that causes early chain termination of viral RNA, was shown to induce SVR rates of 90% in combination with PegIFN and ribavirin triple therapy [43]. Multiple additional inhibitors targeting NS3-4A, NS5B, and NS5A (replication complex protein) have since been developed. Importantly, two-drug DAA combination therapy, of which there are multiple permutations, has been shown to induce SVRs of greater than 90% [44–46]. A list of currently used DAAs is included in Table 13.1.

HCV SVR is associated with significant reductions in all-cause mortality, hepatic fibrosis progression, and hepatocarcinogenesis [60]. In a recent meta-analysis evaluating 25,906 HCV patients treated with IFN-based regimens, the incidence of HCC was 1.5% in patients who achieved SVR compared to 6.2% among nonresponders [61]. Loannou et al. recently performed a meta-analysis evaluating the effect of DAA-associated SVR on HCC incidence. The study included 62,354 patients treated with IFN-only regimens (58%), DAA plus IFN regimens (7%), or

Table 13.1 Current DAAs for HCV and their efficacy, genotype specificity, resistance rates, and combination regiments

DAA type	Drug name	SVR w/ time point	Genotype specificity w/o cirrhosis	Genotype specificity w/ cirrhosis	Resistance rates	Combination regiments
N3/4A protease inhibitor	Glecaprevir	93%/ 12 wk [47]	1–6	1–6	Low	Pibrentasvir
	Grazoprevir	99%/ 12 wk [48]	1a, 1b, 4, 6	1a, 1b, 4	Low	Elbasvir
	Paritaprevir	100%/ 12 wk [49]	1a, 1b, 4	1b, 4	Low	Ombitasvir, dasabuvir, ritonavir
	Simeprevir	79.2%/ 12 wk [50]	1a, 1b, 4		Low	Sofosbuvir
	Voxilaprevir	95%/ 12 wk [51]		3	Low	Sofosbuvir, velpatasvir
NS5A polymerase inhibitor	Daclatasvir	87.4%/ 24 wk [52]	1–4	2, 3	Low	Sofosbuvir
	Elbasvir	97.4%/ 12 wk [53]	1a, 1b, 4, 6	1a, 1b, 4		Grazoprevir
	Ledipasvir	96%/ 12 wk [54]	1a, 1b, 3, 4, 5	1a, 1b, 4	Low	Sofosbuvir
	Ombitasvir	97%/ 12 wk [55]	1a, 1b, 4, 6	1b, 4	<15%	Dasabuvir, ritonavir, paritaprevir
	Pibrentasvir	99.2%/ 12 wk [56]	1–6	1–6	Low	Glecaprevir
	Velpatasvir	99%/ 12 wk [57]	1–6	1–6	<1%	Sofosbuvir
NS5B nucleotide polymerase inhibitor	Sofosbuvir	99%/ 12 wk [58]	1–6	1–6	Low	Simeprevir, voxilaprevir, daclatasvir, ledipasvir, velpatasvir
NS5B non-nucleotide polymerase inhibitor	Dasabuvir	96–100%/ 12 wk [59]	1a, 1b, 2	1b	Low	Ombitasvir, paritaprevir, ritonavir

DAA-only regimens (35%). In all instances, they found an HCC risk reduction of 71–76% in patients who achieved SVR [62]. The risk reduction did not vary by treatment received. However, even among patients who achieve SVR with therapy, the risk for HCC remains elevated compared to healthy controls. The risk of HCC after SVR appears highest in patients with advanced liver fibrosis and diabetes mellitus, and therefore, these patients should be enrolled into HCC screening protocols and additional preventive measures should be considered [63].

HBV Epidemiology and Pathogenesis

As of 2017, 291 million people are infected with hepatitis B (HBV), which is responsible for 887,000 deaths per year globally [64]. HBV is endemic in developing regions including Southeast Asia and Africa, where approximately 8% of the population is infected, which is largely due to a lack of healthcare resources and established neonatal vaccination programs. In contrast, carrier rates in developed Western nations including the United States are below 2%. Two-thirds of patients who develop acute HBV infection exhibit minimal symptoms causing the illness to go undetected, which is a contributing factor to high transmission rates in regions where HBV screening is rarely available [65]. HBV is most frequently vertically transmitted during infancy; therefore, the World Health Organization (WHO) and the Centers for Disease Control (CDC) recommend HBV vaccination within the first 12–24 h after birth. HBV is highly infectious by requiring less than ten viral particles to establish hepatocyte infection [66, 67]. The likelihood of developing chronic infection, and thereby increased cancer risk is related to the age of HBV exposure. In newborns, the risk of chronic HBV infection is 90%, while conversion from acute to chronic infection in adults, who have more well-established immune function, is less than 5% [68]. Chronic HBV is the leading risk factor for HCC development globally (approximately 15-fold increased risk), and approximately 80% of new HCC arise in regions of endemic HBV [69]. Unlike HCV-related HCC, which rarely develops in the absence of cirrhosis, HBV-related HCC can occur in the absence of chronic liver disease, indicating a unique oncogenic mechanism [70].

The pathogenesis for HBV-related HCC is not well established, though is thought to include direct effects from viral DNA integration and translated protein products, as well as indirect effects from secondary hepatic inflammation and fibrosis. Integration of HBV DNA into the host hepatocyte genome is observed in 84.6% of HBV-related HCC [71]. The location and frequency of integrations determines the degree of subsequent genomic instability. Moreover, insertion mutations within tumor suppressor genes have been shown to support aberrant hepatocyte proliferation and survival [71, 72]. Unlike HCV, HBV serum DNA levels directly correlate with risk of HCC development; however, the HBV replication cycle is not directly cytotoxic to hepatic cells [73]. Translation of the HBV genome yields multiple protein products including the viral envelope core, polymerase proteins, and preC and hepatitis B X (HBx) polypeptides [74]. HBx has been associated with multiple oncogenic processes including activation of the Ras-Raf-MAPK pathway, upregulation of miR-181a, and inhibition of p53 and PTEN tumor suppressors [75–77]. Host immune responses to HBV antigens are critical determinants of hepatocellular injury [74]. HBV-induced hepatic inflammation drives local ROS generation and increased hepatocyte oxidative stress, inducing damage to lipids, proteins, and DNA, as well as alteration of multiple cell signaling pathways including MAPK and PI3K. Chronic HBV infection also upregulates hepatic NF-kB and STAT3 signaling and circulating IL-6 levels [78]. These signaling changes stimulate prosurvival gene networks, supporting hepatocyte accumulation of mutational burden while also dampening immune surveillance and thereby increasing the likelihood of malignant transformation.

HBV-Targeted Prevention

Vaccination is the most impactful form of HBV prevention. Establishment of immunity early in life is critical as the likelihood of developing chronic infection, and thus increased HCC risk, is greatest among infants and children. In the United States, initiation of a national neonatal vaccination program in 1981 has led to a substantial drop in the infection rate (9.6 per 100,000 persons in 1982 to 1.1 per 100,000 persons in 2015). The first of 3 vaccine doses are given within 24 h of birth and provides full protection to greater than 90% of infants, children, and adults who receive the entire series [79]. Over 175 countries have now implemented HBV vaccination programs, with the greatest impact occurring in regions with endemic HBV, and it is estimated that over 210 million new chronic infections have been prevented worldwide as of 2015 [80]. For example, Taiwan reported an 80% reduction in HCC incidence upon implementation of a nationwide vaccination program in 1984. Moreover, the childhood/adolescent HCC incidence decreased by 51%, which translated to a 90% reduction in HCC mortality in individuals aged 5–29 years old [81]. However, there are still developing regions with endemic HBV, notably Southeast Asia and Africa, where vaccination programs are lacking, and the burden of HBV-related HCC and mortality remains high.

Multiple challenges hinder successful implementation of birth dose vaccination in low-income and middle-income countries, including high out-of-hospital birth rates, monetary limitations, and healthcare misconceptions regarding vaccine safety [82]. Implementation of successful vaccinations programs in these challenging settings requires understanding of specific regional limitations. For example, Bangladesh, Myanmar, Nepal, and Timor-Leste have low health facility birth rates; therefore, strategies to promote institutional deliveries and out-of-clinic immunizations might provide tailored benefit. Similar principles have been championed in India where a trained healthcare professional is present for approximately 52% of births. Accordingly, programs were instituted to train midwives in vaccination, and standardized neonatal and maternal care protocols were developed, which increased HBV vaccination coverage by twofold [83].

Drug treatment for acute HBV infection is rarely needed for adults, as the vast majority of individuals clear the virus with only mild hepatitis. However, for individuals who develop fulminant hepatitis or chronic infection, antiviral therapy is indicated. Among chronically infected HBV patients, outcomes have improved over the last three decades due to the development of IFN-based therapies and nucleoside/nucleotide analogues (NAs) [84, 85]. Antiviral treatment, regardless of drug type, has been shown to reduce 3-year HCC incidence rate from 4% to 1.5% and 5-year HCC incidence rate from 12% to 5.1% [86]. Blood-based viral load and HBe-Ag titer correlate with viral suppression and future HCC risk; therefore, reductions in these markers are used as surrogates for treatment efficacy [87–89]. Although previous trials have shown that PegIFN/Lamivudine combination therapy is superior to monotherapy for achieving SVR (PegIFN/Lamivudine 57%, Lamivudine 31%, PegIFN 20%), the development of newer and more effective NAs has limited the use of PegIFN, which has a toxic side effect profile [90, 91].

NAs act as viral DNA chain terminators by inhibiting HBV polymerase and reducing HBV replication. Clinically available NAs include lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka), and entecavir (Baraclude). First-generation agents were limited by weak antiviral activity and high susceptibility to drug resistance (76% genotypic resistance after 8 years of treatment for lamivudine and 29% resistance rate after 5 years of adefovir treatment) [92]. Entecavir and tenofovir are newer NA agents that have shown superior efficacy to lamivudine and adefovir, achieving SVR in 95% of patients and regression of cirrhosis in 71– 96% of patients at 3-year follow-up [93, 94]. These drugs are also associated with lower rates of resistance (entecavir resistance between 0.5% and 1.2% after 5 years) [89, 95]. Hosaka et al. showed that among chronically infected HBV patients, the cumulative 5-year HCC incidence was significantly reduced with entecavir monotherapy compared to no treatment (3.7% vs. 13.7%). Furthermore, entecavir reduced HCC incidence by fourfold in cirrhotic patients, who are at greatest risk HCC development [96]. In 2015, the WHO published updated guidelines recommending tenofovir or entecavir as first-line agents [97]. Unfortunately, these newer and more effective NAs are prohibitively expensive for many poor, underdeveloped regions with high rates of endemic HBV infection [98, 99].

Finally, despite improved viral suppression and low resistance rates associated with newer NAs, these medications must be taken indefinitely due to HBV integration into the host genome and continued replication [100]. Only 10% of patients on NA therapy have complete clearance of HBs-Ag after 5 years of treatment [101]. Consequently, although these agents reduce the risk of HCC development, the incidence does not decrease to the level of healthy controls [102]. Therefore, novel approaches targeting multiple steps of the HBV lifecycle are currently under investigation (Table 13.2), including HBV viral entry, formation of covalently closed circular DNA (cccDNA), pregenomic RNA (pgRNA) packing, and virion assembly and secretion [114]. In particular, approaches targeting cccDNA might allow for complete eradication of viral genomic material. In this regard, multiples research teams are investigating the use of CRISPR/Cas9 technology for selective destruction of cccDNA [115, 116]. Other approaches involve epigenetic silencing of cccDNA via HBx targeting and exogenous cytokine therapy to stimulate pathways that drive cccDNA degradation [117, 118].

Metabolic Insult and Disorders

Alcohol Epidemiology and Pathogenesis

Alcoholic cirrhosis affects over 16 million people globally and is responsible for 80% of deaths secondary to liver failure [119]. Of chronic alcohol users, 10–20% will develop cirrhosis, of which 10% will develop HCC [120]. In the United States, approximately 7% of the adult population meets criteria for alcohol abuse/

Table 13.2 Novel therapies for chronic HBV infection

Drug type	Drug name	Clinical trial stage; status	*N*	Results
RNAi therapy	ARB-1467 [103]	Phase 2a/; complete	36	Significant reductions in serum HBsAg and cccDNA levels; dose-dependent relationship in reduction of serum HBsAg
	ARO-HBV [104]	Phase 1b/2a; ongoing	60	N/A
Viral entry inhibitor	Myrcludex B [105]	Phase 1a; complete	36	Drug was well tolerated, pharmacokinetic analysis showed a 2-compartment model, and bioavailability of 85%
Immunotherapy	RO6864018 [106, 107]	Phase 2; complete	31 and 48	N/A
	SB 9200 [108]	Phase 2; ongoing	200	N/A
Capsid inhibitor	NVR 3-778 [109]	Phase 1b; complete	58	Drug was well tolerated with a dose-dependent relationship in reduction HBV DNA
	JNJ56136379 [110, 111]	Phase 2a; ongoing	220 and 84	N/A
	ABI-H0731 [112]	Phase 1b/2a; ongoing	45	N/A
Antisense oligonucleotide therapy	IONIS HBVRx [113]	Phase 2; ongoing	135	N/A

dependence, which is five times higher than the prevalence of HCV [119]. The National Institute on Alcohol Abuse and Alcoholism estimates that 26.9% of Americas greater than 18 years old engage in binge drinking each month [121]. Although alcohol intake information is prone to bias, error, and underreporting, studies have shown that the risk development for HCC increases when daily alcohol intake is chronic and exceeds six drinks per day for more than 10 years [120]. Moreover, heavy drinkers have a 2–23-fold increased risk of death from cirrhosis compared to the general population [122]. Females are more sensitive to alcohol-mediated hepatotoxicity, possibly due to slower rates of alcohol metabolism and greater exposure to hepatotoxic alcohol byproducts [119, 123].

Multiple pathogenic mechanisms contribute to the development of alcohol-related HCC [9]. In hepatocytes, alcohol is initially metabolized by alcohol dehydrogenase (ADH) to acetaldehyde, which in excess quantities can induce hepatocyte DNA adducts [124]. The ADH reaction also increases the NADH/NAD+ ratio, causing an intracellular redox shift that transiently disrupts the TCA cycle, inducing lipogenesis and hepatocyte steatosis [125]. Over time, chronic alcohol intake also activates a supplementary ethanol breakdown pathway called the microsomal

ethanol oxidizing system (MEOS), which generates high quantities of reactive oxygen species, resulting in oxidative stress, lipid peroxidation, and DNA mutations [126]. Collectively, these processes overwhelm the hepatic glutathione system and establish a milieu of hepatocyte death, chronic inflammation, and fibrogenesis, which may ultimately progress to steatohepatitis and eventually cirrhosis and HCC [126]. Finally, when chronic excessive alcohol intake occurs concomitantly with underlying metabolic liver disease or viral infection, the risk of cirrhosis and HCC are magnified presumably due to a synergistic injurious effect on hepatocytes [127, 128].

Alcohol Prevention

Alcohol cessation is the most effective treatment for alcohol-related hepatic injury, which may be accomplished through a combination of psychosocial interventions and pharmacological therapy [129]. Sobriety is challenging to both obtain and maintain, though each subsequent year of abstinence provides additional protection from alcohol-related comorbidities. It has been difficult to quantify the absolute HCC risk reduction with abstinence, which has been estimated at 4–7% per year, though individuals who maintain sobriety for greater than 10 years have a significantly lower risk of developing HCC compared to continued drinkers [130–132].

Critical to the abstinence process is first identifying at risk individuals and providing intervention. Screening and brief interventions (SBIs) in primary care clinics have been shown to be both effective and cost-effective in this regard [133–135]. Multiple intervention styles have been developed and trialed, though in general they involve advice counseling regarding the risks of heavy alcohol consumption by a healthcare professional. Kaner et al. recently published a meta-analysis of 34 studies (n = 15,197) providing moderate-quality evidence that patients receiving brief interventions in the primary care setting consume less alcohol than individuals receiving minimal or no intervention after 1 year [136]. However, the long-term beneficial health implications are more challenging to quantify at a population level. In a recent multi-institutional analysis of SBI practices in multiple European countries, it was observed that screening was executed in only 5.3% of over 6000 participants, which varied greatly by region (mean 1.7% Poland, 9.8% Sweden) [137]. Of the screened positive, interventions were also variably administered, ranging from 59.2% in Catalonia to 94.2% in Poland. The authors concluded that despite policy changes, increased awareness education, and SBI training among healthcare professionals in the studied regions over the last decade, screening and intervention frequency has unfortunately remained largely unchanged, suggesting improvements in this regard are still greatly needed.

Another historically common approach to alcohol cessation has been the use of pharmacologic agents that target the metabolism of ethanol. Disulfiram was the first FDA-approved medication for alcohol abuse, which functions by inhibiting acetaldehyde dehydrogenase, resulting in a buildup of the caustic metabolite, acetalde-

hyde, with alcohol consumption. Elevated acetaldehyde concentrations are associated with nausea, vomiting, headache, and severe physical discomfort. Thus, the function of disulfiram is to associate an undesirable physiologic response with alcohol consumption in order to establish a psychologic aversion [129]. Disulfiram has been shown to effectively reduce relapse rates; however, patient adherence to therapy is poor, limiting its widespread utility.

Currently, there are over 700 clinical trials registered in the United States for the treatment of alcoholism, many of which involve a combined approach of pharmacologic therapy with cognitive behavioral therapy (CBT). Pharmacologic agents under investigation include naltrexone, acamprosate, prazosin, doxazosin, propranolol, psilocybin, varenicline, odansetron, antidepressants (sertraline, escitalopram, duloxetine, mirtazapine), anticonvulsants (topiramate, zonisamide, levetiracetam), antipsychotics (aripriprazole, quetiapine, olanzapine), and analgesics (pregabalin, gabapentin, baclofen, ketamine). Many abstinence interventions are effective for fractional subsets of the population, and one of the challenges going forward is tailoring therapy based on the likelihood of success. Unfortunately, many of these drugs have potential hepatoxicity and are contraindicated in patients with advanced liver disease [129]. Baclofen, a selective $GABA_b$ receptor agonist, is the only pharmacological therapy for alcoholism that has been approved for use in patients with advanced liver disease [138]. Baclofen was shown to be superior to placebo for maintaining abstinence in alcoholics with cirrhosis, which was associated improved liver function tests after 12 weeks of treatment [139]. Baclofen has also been shown to have a HCC preventive effect via induction of hepatocyte cell cycle arrest [140].

NAFLD, Metabolic Syndrome, Obesity Epidemiology, and Pathogenesis

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in developed nations with an estimated prevalence of 30%. NAFLD is defined as hepatic steatosis (HS) in the absence of excessive alcohol consumption. Although it may occur in lean patients, this condition is highly associated with obesity (up to 90% of patients) [141]. NAFLD is also associated with HCC risk, and the incidences of both conditions are rising such that the decreases in virus-related liver cancer might be offset by the rise of fatty liver disease [142, 143].

A subset of NAFLD patients will go on to develop hepatic inflammation in association with steatosis, a condition referred to as nonalcoholic steatohepatitis (NASH). In the United States, NASH effects approximately 3–8% of the population [141]. The transition from NAFLD to NASH is poorly understood, though NASH is a more severe condition in which patients are at increased risk for the development of cirrhosis or HCC. Liver biopsy is required for a diagnosis of NASH, which few patients undergo. Unfortunately, there are currently no reliable noninvasive tests to establish a diagnosis of NASH, and thus the true prevalence of the disease remains unknown, though one study proposed that NASH affects 20–25% of NAFLD patients [144]. Due to these diagnostic limitations, the NAFLD/NASH global bur-

den is likely underestimated. In a more recent meta-analysis, it was estimated that the NASH prevalence among biopsied NAFLD patients is approximately 59.1%, nearly three times higher than what the previous studies have shown [143]. In addition, NASH has been proposed to account for a large proportion of idiopathic or cryptogenic cirrhosis (CC) cases, in which most of the histological hallmarks of NASH are no longer present by the time of presentation, often because patients have lost considerable weight with progressive liver failure. In CC patients who undergo liver transplantation, 25% develop NAFLD and 16% develop NASH within 26 months of transplant [145].

Most patients diagnosed with NASH have stable disease (34–50%) or might even observe improvements in their NAS score (18–29%) over time [146]. However, approximately 9–20% of NASH patients develop an aggressive form of disease that will progress to cirrhosis, and 4–27% go on to develop HCC [147]. Furthermore, common NASH comorbidities including elevated BMI and diabetes have been shown to be independent risk factors for HCC development. Historically, in the United States, the majority of HCC cases have been attributed to chronic HCV infection. However, 15–50% of new HCC cases are idiopathic, and there is reason to believe that NASH accounts for a large proportion of these [148]. A study examining 641 patients with HCC revealed that that clinical features of NASH, including obesity, diabetes, dyslipidemia, and elevated glucose were all significantly associated with CC-related HCC [149]. It is important to note that many of these clinical features, e.g., obesity and diabetes, are themselves independent risk factors for HCC and reports have stated that their coexistence with NASH-related cirrhosis has an additive effect on HCC risk.

The development of HCC in the context of NAFLD/NASH is a complex process with many contributing factors that can be broadly categorized into three main pathogenic mechanisms: cytokine and hormone dysregulation, lipotoxicity, and fibrosis. Obesity is highly prevalent in the NAFLD population and is also an independent risk factor for carcinogenesis by fostering a low-grade chronic inflammatory state throughout the body [150]. Hormonal dysregulation of leptin, adiponectin, and insulin has been implicated with adipose tissue expansion. Leptin is a proinflammatory, proangiogenic, and profibrogenic hormone with growth-promoting effects mediated by JAK/STAT, PI3K/AKT, and ERK signaling pathways [151]. Adipose content and leptin levels are positively correlated, and leptin has been shown to activate hepatic Kupffer and stellate cells, both of which have been connected to NAFLD/NASH fibrotic disease progression and HCC development [152]. In contrast, adiponectin is an anti-inflammatory hormone that is reduced in NAFLD. Low levels of adiponectin reduce its regulatory impact on inflammatory cell signaling, the mTOR mitogenic pathway, and angiogenesis, all of which promote hepatic oncogenesis [153]. Finally, insulin resistance and subsequent hyperinsulinemia are commonly observed in states of obesity and NAFLD. Elevated insulin levels upregulate the production of IGF1, which stimulates hepatic cellular proliferation and inhibits apoptosis through downstream activation of various oncogenic pathways including MAPK, PI3K/AKT, and VEGF [154]. Taken together, NAFLD-associated hormonal dysregulation supports proinflammatory and prosurvival sig-

naling while downregulating checkpoint signals, establishing an environment in which hepatocyte malignant transformation is likely to occur unchecked.

Hepatocytes are capable of de novo lipogenesis (DNL), both as a means of lipid production for other tissues and for hepatocyte metabolism. Aberrantly elevated DNL, which is observed in NASH, has recently been implicated as a pathogenic mechanism that contributes to HCC carcinogenesis. Specifically, it has been postulated that upregulated DNL might provide necessary energy for the growing microenvironment of pre-cancerous lesions [155, 156]. Moreover, tumor mRNA expression of prolipogenic genes such as acetyl CoA carboxylase and fatty acid synthase correlates with cell proliferation rates and poor HCC prognosis [156, 157]. Another consequence of aberrant DNL is lipotoxicity, in which excessive mitochondrial B-oxidation and degradation of free fatty acid intermediates lead to reactive oxygen species generation, subsequently inducing ER stress, inflammation, and DNA damage. Obese mouse models have demonstrated that ROS production increases with hepatic fatty infiltration and is associated with the development of hepatic hyperplasia and dysplasia, both of which precede development of invasive malignancy [158, 159]. Lipid accumulation also increases lipid breakdown products, some of which can cause direct damage to hepatocytes. Trans-4-hydroxy-2-noneal is a byproduct of lipid peroxidation and has been shown to cause mutations within the TP53 tumor suppressor gene, a common mutation involved in over 50% of HCC cases [160]. NRF1 is an essential transcription factor that mediates oxidative stress in hepatocytes, which is downregulated in animal models of NASH. Decreased NRF1 expression correlated with hepatic steatosis, inflammation, fibrosis, and HCC development [161].

Persistent lipotoxic hepatocyte injury is associated with constitutive activation of tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6), driving chronic hepatic inflammation [150]. TNFα is a potent activator of multiple pro-oncotic pathways that involve mTOR, JNK, and NF-κB [162]. IL-6 is an inflammatory cytokine that exhibits antiapoptotic and cell-proliferative phenotypes through the activation of STAT3, a transcriptional factor linked to malignant transformation and HCC aggressiveness [163, 164]. Experimental mouse models of dietary obesity have been linked to the activation of IL-6, TNFα, and their associated oncogenic signaling pathways, which was associated with increased HCC risk [162, 163]. Chronic hepatic inflammation is also associated with hepatic stellate cell activation and fibrogenesis, which may progress to cirrhosis. It is unclear whether hepatic fibrosis is an independent risk factor for HCC development, or rather that fibrosis correlates with advanced parenchymal disease. However, more than one-third of the NASH-related HCC occurs in the absence of cirrhosis [144]. Noncirrhotic HCC patients are typically older, suggesting there may be differences in disease biology [165].

NASH Prevention

A major challenge in engineering therapies for metabolic disorders is creating a treatment that targets a multifaceted disease. Although multiple medications targeting lipid homeostasis and glucose metabolism are currently in use to treat obesity

and diabetes, there are currently no FDA-approved drugs that specifically target NASH. To date, the only accepted treatments for NASH are weight loss by lifestyle modification or bariatric surgery. In a recent analysis of patients treated with weight loss interventions, individuals capable of losing at least 5% of their body weight showed a significant reduction of hepatic steatosis. Weight loss of greater than 7% was associated with improvement in NALFD Activity (NAS) score, and weight loss greater than 10% was associated with improvement in histologic features of NASH, including portal inflammation and fibrosis [166, 167]. In addition, the type of diet appears to be less important than sustained weight loss for the resolution of NAFLD [168]. Although the majority of obese and NAFLD patients participate in minimal physical activity, large randomized control trials assessing the direct effect of exercise on NASH are missing. Retrospective evidence has suggested that exercising five times per week for a minimum of 10 min was linked to decreases in new fatty liver deposition and improvements in existing liver disease [169].

Bariatric surgery has emerged as an effective alternative weight loss treatment across the spectrum of NAFLD/NASH patients. A meta-analysis of 15 studies researching the effects of bariatric surgery in NASH showed improvements in steatosis (91.6%), steatohepatitis (81.3%), fibrosis (65.5%), and disease resolution (69.5%) after surgery [170]. Two subsequent prospective studies in obese patients with NASH saw disease resolution in 85% of cases at 1 year after surgery and 69.7% after 5 years [171, 172]. Despite evidence to support its efficacy, bariatric surgery is currently restricted to patients with a body mass index ≥40 or ≥35 with obesity-related comorbidities. Most patients who receive bariatric surgery retain weight loss rates between 14% and 25% below their preoperative weight after 10 years, and they show improvements in diabetes, insulin resistance, and cardiovascular events [173–175]. Given the strong association between weight loss and improvement in NASH severity, expanding indications for bariatric surgery to include NASH in patients with elevated BMI might be of value. The hesitation in doing so is partly due to a lack of high-level evidence that establishes a direct connection between bariatric surgery and NASH [176]. The effect of bariatric surgery on hepatic fibrosis is also controversial. Initial studies suggested that hepatic fibrosis increased after surgery due to increases in proinflammatory cytokines [177]. However, recent evidence has shown that 80–95% of patients see no change or regression in their hepatic fibrosis status at 5 years post-op [172]. This may be in part due to a reduction in hepatic profibrogenic cytokine gene expression, thereby attenuating hepatitis and subsequent fibrosis [178]. Finally, there is concern regarding the risk of surgery in patients with intrinsic liver disease. A recent study by Jan et al. showed no difference in complication rate after bariatric surgery in patients with compensated cirrhosis compared to those without the evidence of liver disease [179–182]. However, decompensated cirrhosis remains a contraindication to bariatric surgery [180, 182, 183].

Similar to bariatric surgery, it is thought that medications targeting metabolic disorders may have secondary benefits on HCC prevention. Metformin, an insulin sensitizer used as first-line treatment for hyperglycemia and noninsulin-dependent type 2 diabetes, is currently under investigation as a possible HCC preventative

agent. Retrospective studies and pooled analysis have shown that metformin decreased HCC incidence up to 50% when compared to observation alone [184, 185]. However, RCT examining the effects of metformin on HCC development does not support the claim that metformin is superior to other antidiabetic agents in reducing HCC [186].

Pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARδ) activator, is another diabetes medication that showed promising results as an anti-NASH agent. Several RCTs in diabetic and nondiabetic populations consistently showed improvements in NASH histological parameters (steatosis, parenchymal and lobular inflammation, hepatocyte ballooning, and NAS score), which are believed to indirectly reduce the risk of HCC [187–191]. Despite these promising results, multiple studies have associated pioglitazone with an unfavorable side effect profile including fluid retention and weight gain, limiting its widespread use [192–194].

Statins are commonly used to reduce the risk for cardiovascular disease and diabetes; however, there has been evidence to support their use as a chemopreventive agent in a variety of cancers [195]. Statins reduce blood cholesterol by inhibiting HMG-CoA reductase, which consequently decreases the production of mevalonate pathway metabolites, a pathway recently implicated in cell growth and cancer transformation [196]. Singh et al. investigated the effects of statins on the risk of HCC by conducting a meta-analysis that included 26 randomized control trials and over 1.4 million patients [197]. They found that statin use was associated with a 37% decrease in the risk of HCC after adjusting for confounding variables. Furthermore, a recent retrospective study of nearly 10,000 patients showed that the beneficial effect of statin use on HCC chemoprevention was greater in patients with diabetes or cirrhosis [198]. However, one RCT did not demonstrate a significant difference in HCC incidence between statin and placebo groups [199]. Further investigation regarding the mechanism of statin-mediated anticancer effects remains ongoing.

More recently, a plethora of drugs designed to specifically target NASH pathogenesis are in clinical development (Table 13.3). Given the complex pathophysiology of NASH, these drugs have been designed to have either metabolic, antisteatotic, anti-inflammatory, or antifibrotic effects. It is expected that drugs that can impede several of these disease mechanisms or drug combinations that can successfully inhibit multiple pathways will have the most beneficial effects for patients. Obeticholic acid (OCA) was the first such drug designed for NASH patients and is a Farnesoid X Receptor agonist that functions to reduce the conversion of cholesterol to bile acids in the liver, which has been associated with reductions in cholestasis, hepatic inflammation, hepatocyte injury, and HCC development. In a phase II clinical trial, OCA was associated with significant reductions in histologic markers of NASH [200, 201]. OCA is being subsequently investigated in a phase III clinical trial (REGENERATE; NCT02548351), examining the long-term effects of OCA on fibrosis and mortality in NASH patients. In the 18-month interim analysis, OCA 25 mg once daily met the primary endpoint of fibrosis improvement (≥1 stage) without worsening NASH.

Table 13.3 Clinical trials for the pharmacological therapies for NASH

Drug type	Drug name	Clinical trial stage; status	Primary endpoints	*N*	Duration	Results
PPAR-gamma agonist	MSDC-0602K	Phase II; ongoing	Hepatic histological improvements in NAS	380	12 months	N/A
PPAR-alpha/gamma agonist	Saroglitazar	Phase II; ongoing	Percentage change from baseline in serum ALT	104	16 weeks	N/A
PPAR-alpha/delta agonist	Elafibranor	Phase III; ongoing	1. NASH resolution without worsening of fibrosis 2. Long-term outcomes: mortality, cirrhosis, and liver-related clinical outcomes	2000	72 weeks, 4 years	N/A
Pan-PPAR agonist	IVA337	Phase II; ongoing	Improvement in SAF score	225	24 weeks	N/A
GLP-1 receptor agonist	Semaglutide	Phase II; ongoing	NASH resolution without worsening of fibrosis	228	72 weeks	N/A
SGLT-2	Empagliflozin	Phase IV; ongoing	Change in liver triglyceride concentrations	72	24 weeks	N/A
SCD inhibitor	Aramchol	Phase II, III; completed	Change in liver triglyceride concentrations	247	52 weeks	N/A
FXR ligand	Obeticholic acid	Phase III; ongoing	1. NASH resolution without worsening of fibrosis 2. Rates of death, liver transplantation, HCC, ascites, cirrhosis, or hospitalizations 3. MELD score	2370	18 months	N/A
Non-bile acid synthetic FXR agonist	GS-9674	Phase II; completed	1. Proportion of participants experiencing adverse events 2. Proportion of participants with lab abnormalities	140	24 weeks	N/A
	LMB763	Phase II; ongoing	1. Adverse event profile and safety endpoints 2. Change in transaminase levels	192	12 weeks	N/A
	LJN452	Phase II; ongoing	1. Adverse event profile 2. Change in transaminase levels 3. Change in steatosis from baseline	345	12 weeks	N/A

Thyroid hormone receptor beta agonist	MGL-3196	Phase II; ongoing	Change of hepatic fat fraction from baseline	125	12 weeks	N/A
ASK-1 inhibitor	Selonsertib	Phase III; ongoing	1. Proportion of participants achieving >1 stage improvement in fibrosis staging 2. Event-free survival estimated by time to the first clinical event	808	48 weeks, 240 weeks	N/A
Caspase inhibitor	Emricasan	Phase II; ongoing	Fibrosis improvements without worsening of steatohepatitis	330	72 weeks	N/A
Vascular adhesion protein-1 inhibitor	BI 1467335	Phase II; ongoing	Plasma amine oxidase copper-containing 3 (AOC3) activity relative to baseline measured at 24 h post dose	147	12 weeks	N/A
TLR-4 antagonist	JKB-121	Phase II; completed	1. Rate of adverse events 2. Change from baseline in hepatic fat content 3. Change from baseline in serum ALT levels 4. Time to remission	66	24 weeks	No difference between JKB-121 and placebo in reduction of hepatic fat content or ALT levels
CCR2/5 antagonist	Cenicriviroc	Phase III; ongoing	1. Superiority of CVC to placebo on liver histology 2. Superiority of CVC compared to placebo on cirrhosis, liver-related clinical outcomes, and mortality	2000	12 months	N/A
Galectin-3 inhibitor	GR-MD-02	Phase II; completed	Efficacy on reducing hepatic venous pressure gradient (HVPG) as a measure of portal pressure compared to placebo in NASH cirrhotic patients HVPG reduction was defined by absolute reduction of >2 mmHg in HVPG from baseline or >20% reduction of HVPG from baseline	162	1 year	Statistical significant reduction in HVPG in patients using GR-MD-02 vs placebo (44% vs 15%; $p = 0.02$)
Fibroblast growth factor 21	BMS-986036	Phase II; completed	1. Change in hepatic fat fraction 2. Safety, as measured by adverse events 3. Safety, as measured by clinical laboratory tests 4. Safety, as measured by vital signs 5. Safety, as measured by electrocardiograms 6. Safety, as measured by physical examinations	202	16 weeks	1. Significant reduction of hepatic fat fraction (6.8% vs 1.3%; $p = 0.0004$) 2. Favorable safety profile with no adverse events and no deaths

Elafibranor is a dual PPARα/δ agonist that functions by decreasing serum glucose and triglyceride levels and has also been shown to have anti-inflammatory properties. Elafibranor was studied in a phase IIa clinical trial (GOLDEN; NCT01694849) of NASH patients in which it was associated with reductions in hepatocyte ballooning, lobar inflammation, fibrosis staging, and cardiometabolic risk profile [202]. These improvements were most evident in patients with NAS scores ≥4. Importantly, unlike some PPARα/δ agonists, there has been no evidence to suggest that Elafibranor causes weight gain. Elafibranor is currently in a phase III clinical trial (RESOLVE-IT; NCT02704403) investigating its impact on fibrosis staging after 72 weeks of treatment, as well as rates of mortality, cirrhosis, and liver-related comorbidities up to 4 years after therapy.

Cenicriviroc is a CCR2/5 inhibitor which efficiently inhibits monocyte infiltration and chemokine activation. CCR2 is upregulated in fibrotic human livers alongside an accumulation of monocyte-derived phagocytes [203]. In obese NASH patients, proportions of CCR2+ macrophages in visceral adipose tissue are associated with histological disease severity [204]. Clinical trials testing cenicriviroc in the NASH population showed decreases in hepatic fibrosis and slowing NASH progression [205]. Cenicriviroc is in a phase III clinical trial (AURORA; NCT03028740) examining its impact on fibrosis staging after 12 months of treatment and its effect on cirrhosis rates, liver related clinical outcomes, and mortality 5 years after therapy.

Selonsertib is an apoptosis signal-regulating kinase 1 (ASK-1) inhibitor designed to treat NASH. Activation of ASK-1 is normally achieved by TNF-alpha or cellular stress (oxidative or ER), leading to activation of p38/JNK pathway and subsequent hepatic fibrosis and apotosis [206]. In a murine model of NASH, selonsertib improved metabolic parameters (serum cholesterol and bile acid), hepatic steatosis, inflammation, and fibrosis [207]. Moreover, combination therapy with simtuzumab, a lysyl oxidase-like molecule 2 (LOXL2) inhibitor, potentiated the antifibrotic effects of anti-ASK and anti-LOXL2 monotherapy in mice [208]. This evidence warranted a phase II clinical trial testing the effect of selonsertib in NASH which showed significant reduction in hepatic fibrosis, lobular inflammation, and serum biomarkers for apoptosis (cytokeratin-18 M30) and necrosis (cytokeratin-18 M65) after 24 weeks of treatment [209]. Selonsertib is being evaluated in a phase III clinical trial (STELLAR 4; NCT03053063) evaluating its ability to improve hepatic fibrosis and survival in patients with NASH-related compensated cirrhosis, although the primary endpoint for fibrosis was not met.

Molecular Targeted Chemoprevention

HCC-risk-driving molecular pathway dysregulation shared across multiple etiologies could be utilized for molecular targeted chemoprevention that benefits broader patient populations. One example is epidermal growth factor (EGF) pathway, which plays a role in hepatocarcinogenesis in cirrhosis caused by various etiologies [8]. Elevated EGF expression is a key feature in gene signatures that are predictive of

progressive cirrhosis, HCC development, and death in patients with cirrhosis [210–216]. A single-nucleotide polymorphism (SNP) in the EGF gene (rs4444903, 61∗G allele) is associated with increased hepatic EGF expression and elevated risk of developing HCC across various etiologies and patient race/ethnicity [214, 217]. Transgenic mice with liver-specific overexpression of EGF develop HCC, supporting its functional relevance [212]. In preclinical studies, inhibition of EGF receptor (EGFR) activation by the small molecule tyrosine kinase inhibitor, erlotinib, effectively inhibits stellate cell activation, hepatic fibrosis, and development of HCC [211]. In a subset of animals, reversal of fibrosis was observed in paired longitudinal liver tissue analysis. Similar results were observed in preclinical studies with another small molecular EGFR inhibitor, gefitinib, though the observed effect was attributed to direct antitumor effects of gefitinib [212]. Genetic knockout of the signaling in macrophages in the liver similarly suppressed HCC development in mice [218]. These results collectively suggest that activation of the EGF pathway in hepatic stromal cells contribute to creating carcinogenesis-supporting tissue microenvironment, and can be therapeutically antagonized to achieve HCC chemoprevention. Based on these observations, erlotinib is currently under clinical evaluation for its effectiveness in reducing hepatic fibrogenesis and preventing HCC (NCT02273362). An interim analysis in the phase I trial has identified the minimum effective dose of 25 mg/day that suppresses hepatic phospho-EGFR levels. At the dose, which is one-sixth of the oncology dose, no adverse effect was observed. This is an encouraging observation, supporting the use of low-dose erlotinib as a safe orally available HCC chemoprevention. A recent large cohort study suggested that use of aspirin but not other NSAIDs is associated with lower probability of incident HCC when 650 mg or more per week is taken for 5 years or more [219]. In support of this epidemiological observation, anti-platelet effect of aspirin was found to inhibit platelet aggregation and reduced HCC incidence in NASH mouse model [220].

Tertiary Prevention

Curative treatment options for HCC are limited due to the lack of effective chemotherapy and radiotherapy. Therefore, tumor resection and orthotopic liver transplantation (OLT) are the only options for long-term cure. Although advances in these surgical procedures have significantly improved perioperative morbidity, over 70% of patients still develop intrahepatic recurrence within 5 years of their first hepatectomy [221]. HCC recurrence rates among liver transplant recipients is varied between centers (6.4–56.5%; 5-year recurrence rates); however, tumor size, nodule count, vascular invasion, presence of cirrhosis, and tumor grade have emerged as the most clinically predictive characteristics for recurrence [222].

Tertiary HCC prevention aims to prevent cancer recurrence in patients curatively treated for initial cancer as adjuvant therapy [24]. Various strategies to prevent the recurrence of HCC have been tried, such as vitamin K2, retinoids, and systemic chemotherapy, although none were proven to be effective when tested in large-scale randomized control trials [223–225]. Interferon is the most widely used adjuvant

tertiary prevention therapy, although evidence to support its effectiveness in reducing recurrence in conflicting [226]. Creating tertiary prevention strategies for HCC is a major challenge due to its intrinsic chemoresistance which has been linked to various mechanisms including overexpression of drug efflux pumps (MDR1 and MRP2), enhanced DNA repair mutations (ERCC-1, FENs, Chk2, ATM, APE1), impairment of apoptotic machinery (CD95, FADD, FLICE), and activation of cell survival signaling (Hedgehog, Hippo, Wnt/beta-catenin) [227]. Unlike other cancers where metastasis is the primary method for recurrence, HCC has a heightened risk of de novo carcinogenesis especially patients with cirrhosis or viral infection. Moreover, the majority of HCC recurrence occurs in the liver remnant, making it nearly impossible to differentiate its origin. For these reasons, it has been difficult to study and find an agent that can inhibit both HCC recurrence mechanisms.

A multityrosine kinase inhibitor, sorafenib, inhibits cell proliferation and angiogenesis in murine models and therefore may reduce HCC recurrence [228]. A phase III clinical trial (STORM) showed sorafenib did not reduce recurrence-free survival compared to control and deemed it ineffective as an adjuvant intervention for HCC following resection or ablation [229]. Although HCC is not normally considered an immunogenic tumor, it has been reported that patients with higher levels of lymphocytes within their tumors have longer survival rates and are at lower risk of recurrence [230, 231]. Patients expressing higher numbers of tumor-associated antigens (TAA) also had better survival rates than those with fewer TAAs indicating a role for a patient's own immune system in fending off HCC-related comorbidities [231]. These results provide the rationale for immunotherapy as a tertiary prevention against HCC recurrence. Immune checkpoint blockade by anti-PD-1 antibodies, nivolumab and (CHECKMATE 040; NCT01658878) and pembrolizumab (KEYNOTE-224; NCT02702414), demonstrated objective response rates of up to 20% in the setting of oncology treatment [232, 233]. Based on the result, immune checkpoint inhibition is under evaluation as adjuvant therapy following HCC resection or ablation (Checkmate 9DX; NCT03383458). A combination of a VEGF inhibitor, bevacizumab, and an anti-PD-L1 antibody, atezolizumab, yielded a high objective response rate of 32% and a 6-month progression free survival of 65% (NCT02715531) [233]. This suggests that combination therapy may also serve as adjuvant therapy in HCC. Cytokine-induced killer (CIK) cell-based therapy is one of the promising adjuvant immunotherapies tested in HCC. CIK cells are a mixture of T cells (CD3+/CD56+ cells and CD3+/CD56− T cells and CD3−/CD56+ natural killer cells) that are ex vivo expanded using cytokines. Preclinical studies showed CIK cells have multiple favorable characteristics including potent in vitro HCC-targeted damage, ability to localize within hepatic cancer mass in vivo, and no major side effects with repeated therapies [234–236]. These encouraging preclinical studies allowed for the phase III clinical trial showing that activated CIK treatment in patients previously treated with HCC surgical resection, radiofrequency ablation, or percutaneous ethanol injection extended median recurrence-free and overall survival compared to placebo [237]. These results have continued to hold as Lee and colleagues recently reported significant improvements in 5-year recurrence-free and overall survival rates [238]. Other immunotherapy treatments attempt to treat HCC using more targeted forms of therapy including utilizing

CAR-T cells, HCC vaccines, and altering immune checkpoint inhibitors. These types of therapies have been designed to treat existing HCC; however, it is conceivable for them to be used to attenuate the risk of recurrence due to their anticarcinogenesis effects [239].

Conclusions and Future Directions

Given the lack of successful treatment options for HCC, prevention should be given more attention. Primary prevention strategies have already shown remarkable success in reducing HCC incidence. For example, after decades of research, DAAs have emerged as a promising approach to reduce HCC incidence in the majority of HCV patients. Risk of HCC does still persist especially in those patients with advanced fibrosis at the time of treatment, so screening strategies will need to be implemented for these patients. While a vaccine for HCV remains elusive, efforts in this area remain ongoing and could offer an additional solution for cure. Likewise, HBV vaccination has proven to be a very effective strategy for reducing HCC incidence in places where HBV is endemic. Unfortunately, socioeconomic concerns have lagged behind the science in these instances as not all people at risk for HBV infection have access to adequate healthcare with HBV vaccination programs and similarly not all HCV patients currently have access to DAAs given their high cost. Solutions for these problems, like midwife vaccination programs, will need to be continually evaluated in the coming years.

It is not as clear whether alcohol cessation or changes in eating habits and weight loss will be as sustainable in reducing HCC risk as a result of ASH and NASH, respectively. Alternative solutions like liver transplantation and bariatric surgery exist but also come with a high socioeconomic cost. A plethora of trials are underway to evaluate new drugs that target different pathways in the disease pathogenesis including insulin resistance, de novo lipogenesis, inflammation, and fibrogenesis, but more efforts should be deployed for repurposing current drugs, like metformin, which have been associated with decreased HCC incidence in retrospective analyses.

Given the long duration between exposure to insult and development of primary HCC, investigation of preventative therapies will most likely occur in the tertiary setting and/or secondary setting with enrichment of high-risk cirrhosis population [8]. Trial designs could include DNA sequencing to verify that recurrent tumors are in fact de novo cancers as opposed to regrowth of a previous unsuccessfully treated tumor. In addition, both invasive, like gene signatures, and noninvasive biomarkers, like serum proteins or DNA, could be evaluated for their ability to predict successful therapies. Such biomarkers would be instrumental in the subsequent evaluation of promising therapies into the primary setting.

In conclusion, given the readily identifiable population at risk, HCC prevention is an achievable goal as supported by numerous successful programs to date. Given the increasing incidence and the lack of effective treatments, more efforts in HCC prevention should be undertaken to improve the prognosis of this deadly disease.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94(2):153–6.
2. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27. https://doi.org/10.1056/NEJMra1001683.
3. Goldberg DS, Valderrama A, Kamalakar R, Sansgiry SS, Babajanyan S, Lewis JD. Hepatocellular carcinoma surveillance among cirrhotic patients with commercial health insurance. J Clin Gastroenterol. 2016;50(3):258–65. https://doi.org/10.1097/MCG.0000000000000411.
4. Palmer LB, Kappelman MD, Sandler RS, Hayashi PH. Surveillance for hepatocellular carcinoma in a Medicaid cirrhotic population. J Clin Gastroenterol. 2013;47(8):713–8. https://doi.org/10.1097/MCG.0b013e318286fd97.
5. Davila JA, Henderson L, Kramer JR, Kanwal F, Richardson PA, Duan Z, et al. Utilization of surveillance for hepatocellular carcinoma among hepatitis C virus-infected veterans in the United States. Ann Intern Med. 2011;154(2):85–93. https://doi.org/10.7326/0003-4819-154-2-201101180-00006.
6. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. https://doi.org/10.3322/caac.21262.
7. Choo SP, Tan WL, Goh BK, Tai WM, Zhu AX. Comparison of hepatocellular carcinoma in Eastern versus Western populations. Cancer. 2016;122(22):3430–46. https://doi.org/10.1002/cncr.30237.
8. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. 2018;68(3):526–49. https://doi.org/10.1016/j.jhep.2017.09.016.
9. Hoshida Y, Fuchs BC, Tanabe KK. Prevention of hepatocellular carcinoma: potential targets, experimental models, and clinical challenges. Curr Cancer Drug Targets. 2012;12(9):1129–59.
10. Luo Z, Li L, Ruan B. Impact of the implementation of a vaccination strategy on hepatitis B virus infections in China over a 20-year period. Int J Infect Dis. 2012;16(2):e82–8. https://doi.org/10.1016/j.ijid.2011.10.009.
11. Magnussen A, Parsi MA. Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol. 2013;19(10):1508–12. https://doi.org/10.3748/wjg.v19.i10.1508.
12. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol. 2017;23(47):8263–76. https://doi.org/10.3748/wjg.v23.i47.8263.
13. Kim NG, Nguyen PP, Dang H, Kumari R, Garcia G, Esquivel CO, et al. Temporal trends in disease presentation and survival of patients with hepatocellular carcinoma: a real-world experience from 1998 to 2015. Cancer. 2018;124(12):2588–98. https://doi.org/10.1002/cncr.31373.
14. Traussnigg S, Kienbacher C, Halilbasic E, Rechling C, Kazemi-Shirazi L, Hofer H, et al. Challenges and Management of Liver Cirrhosis: practical issues in the therapy of patients with cirrhosis due to NAFLD and NASH. Dig Dis. 2015;33(4):598–607. https://doi.org/10.1159/000375353.
15. Perricone G, Duvoux C, Berenguer M, Cortesi PA, Vinaixa C, Facchetti R, et al. Delisting HCV infected liver transplant candidates who improved after viral eradication: outcome 2 years after delisting. Liver Int. 2018;38(12):2170–7. https://doi.org/10.1111/liv.13878.
16. Simonetti RG, Camma C, Fiorello F, Politi F, D'Amico G, Pagliaro L. Hepatocellular carcinoma. A worldwide problem and the major risk factors. Dig Dis Sci. 1991;36(7):962–72.
17. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5 Suppl 1):S35–50.
18. Haukeland JW, Lorgen I, Schreiner LT, Frigstad SO, Brandsaeter B, Bjoro K, et al. Incidence rates and causes of cirrhosis in a Norwegian population. Scand J Gastroenterol. 2007;42(12):1501–8. https://doi.org/10.1080/00365520701478014.

19. Ding J, Wang H. Multiple interactive factors in hepatocarcinogenesis. Cancer Lett. 2014;346(1):17–23. https://doi.org/10.1016/j.canlet.2013.12.024.
20. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529–38. https://doi.org/10.1016/j.jhep.2006.05.013.
21. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med. 1999;340(10):745–50. https://doi.org/10.1056/NEJM199903113401001.
22. Tsai WL, Chung RT. Viral hepatocarcinogenesis. Oncogene. 2010;29(16):2309–24. https://doi.org/10.1038/onc.2010.36.
23. Branda M, Wands JR. Signal transduction cascades and hepatitis B and C related hepatocellular carcinoma. Hepatology. 2006;43(5):891–902. https://doi.org/10.1002/hep.21196.
24. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. J Hepatol. 2014;61(1 Suppl):S79–90. https://doi.org/10.1016/j.jhep.2014.07.010.
25. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009;27(9):1485–91. https://doi.org/10.1200/JCO.2008.20.7753.
26. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. Ann Intern Med. 1999;131(3):174–81.
27. Ishiguro S, Inoue M, Tanaka Y, Mizokami M, Iwasaki M, Tsugane S, et al. Impact of viral load of hepatitis C on the incidence of hepatocellular carcinoma: a population-based cohort study (JPHC Study). Cancer Lett. 2011;300(2):173–9. https://doi.org/10.1016/j.canlet.2010.10.002.
28. Lee MH, Yang HI, Lu SN, Jen CL, Yeh SH, Liu CJ, et al. Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. J Clin Oncol. 2010;28(30):4587–93. https://doi.org/10.1200/JCO.2010.29.1500.
29. Gravitz L. Introduction: a smouldering public-health crisis. Nature. 2011;474(7350):S2–4. https://doi.org/10.1038/474S2a.
30. Smith BD, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Teo CG, et al. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. MMWR Recomm Rep. 2012;61(RR-4):1–32.
31. Alter MJ, Hadler SC, Judson FN, Mares A, Alexander WJ, Hu PY, et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. JAMA. 1990;264(17):2231–5.
32. Rein DB, Wittenborn JS, Weinbaum CM, Sabin M, Smith BD, Lesesne SB. Forecasting the morbidity and mortality associated with prevalent cases of pre-cirrhotic chronic hepatitis C in the United States. Dig Liver Dis. 2011;43(1):66–72. https://doi.org/10.1016/j.dld.2010.05.006.
33. Ray RB, Steele R, Meyer K, Ray R. Transcriptional repression of p53 promoter by hepatitis C virus core protein. J Biol Chem. 1997;272(17):10983–6.
34. Cho J, Baek W, Yang S, Chang J, Sung YC, Suh M. HCV core protein modulates Rb pathway through pRb down-regulation and E2F-1 up-regulation. Biochim Biophys Acta. 2001;1538(1):59–66.
35. Bandiera S, Billie Bian C, Hoshida Y, Baumert TF, Zeisel MB. Chronic hepatitis C virus infection and pathogenesis of hepatocellular carcinoma. Curr Opin Virol. 2016;20:99–105. https://doi.org/10.1016/j.coviro.2016.09.010.
36. Irshad M, Dhar I. Hepatitis C virus core protein: an update on its molecular biology, cellular functions and clinical implications. Med Princ Pract. 2006;15(6):405–16. https://doi.org/10.1159/000095485.
37. Lerat H, Higgs M, Pawlotsky JM. Animal models in the study of hepatitis C virus-associated liver pathologies. Expert Rev Gastroenterol Hepatol. 2011;5(3):341–52. https://doi.org/10.1586/egh.11.14.

38. Hara Y, Yanatori I, Ikeda M, Kiyokage E, Nishina S, Tomiyama Y, et al. Hepatitis C virus core protein suppresses mitophagy by interacting with parkin in the context of mitochondrial depolarization. Am J Pathol. 2014;184(11):3026–39. https://doi.org/10.1016/j.ajpath.2014.07.024.
39. Bruno S, Shiffman ML, Roberts SK, Gane EJ, Messinger D, Hadziyannis SJ, et al. Efficacy and safety of peginterferon alfa-2a (40KD) plus ribavirin in hepatitis C patients with advanced fibrosis and cirrhosis. Hepatology. 2010;51(2):388–97. https://doi.org/10.1002/hep.23340.
40. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011;364(13):1207–17. https://doi.org/10.1056/NEJMoa1009482.
41. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364(25):2405–16. https://doi.org/10.1056/NEJMoa1012912.
42. Manns M, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, et al. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2014;384(9941):414–26. https://doi.org/10.1016/S0140-6736(14)60538-9.
43. Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med. 2013;368(20):1878–87. https://doi.org/10.1056/NEJMoa1214853.
44. Kowdley KV, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med. 2014;370(20):1879–88. https://doi.org/10.1056/NEJMoa1402355.
45. Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet. 2014;384(9956):1756–65. https://doi.org/10.1016/S0140-6736(14)61036-9.
46. Feld JJ, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, et al. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med. 2014;370(17):1594–603. https://doi.org/10.1056/NEJMoa1315722.
47. Puoti M, Foster GR, Wang S, Mutimer D, Gane E, Moreno C, et al. High SVR12 with 8-week and 12-week glecaprevir/pibrentasvir therapy: an integrated analysis of HCV genotype 1-6 patients without cirrhosis. J Hepatol. 2018;69(2):293–300. https://doi.org/10.1016/j.jhep.2018.03.007.
48. Roth D, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H Jr, et al. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. Lancet. 2015;386(10003):1537–45. https://doi.org/10.1016/s0140-6736(15)00349-9.
49. Hezode C, Asselah T, Reddy KR, Hassanein T, Berenguer M, Fleischer-Stepniewska K, et al. Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naive and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. Lancet. 2015;385(9986):2502–9. https://doi.org/10.1016/s0140-6736(15)60159-3.
50. Forns X, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, et al. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. Gastroenterology. 2014;146(7):1669–79.e3. https://doi.org/10.1053/j.gastro.2014.02.051.
51. Jacobson IM, Lawitz E, Gane EJ, Willems BE, Ruane PJ, Nahass RG, et al. Efficacy of 8 weeks of sofosbuvir, velpatasvir, and voxilaprevir in patients with chronic HCV infection: 2 phase 3 randomized trials. Gastroenterology. 2017;153(1):113–22. https://doi.org/10.1053/j.gastro.2017.03.047.

52. Izumi N. Efficacy of daclatasvir in hepatitis C virus. Expert Rev Anti-Infect Ther. 2014;12(9):1025–31. https://doi.org/10.1586/14787210.2014.942282.
53. Toyoda H, Atsukawa M, Takaguchi K, Senoh T, Michitaka K, Hiraoka A, et al. Real-world virological efficacy and safety of elbasvir and grazoprevir in patients with chronic hepatitis C virus genotype 1 infection in Japan. J Gastroenterol. 2018;53(12):1276–84. https://doi.org/10.1007/s00535-018-1473-z.
54. Bourliere M, Bronowicki JP, de Ledinghen V, Hezode C, Zoulim F, Mathurin P, et al. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). Lancet Infect Dis. 2015;15(4):397–404. https://doi.org/10.1016/s1473-3099(15)70050-2.
55. Ahmed H, Abushouk AI, Menshawy A, Mohamed A, Negida A, Loutfy SA, et al. Safety and efficacy of ombitasvir/paritaprevir/ritonavir and dasabuvir with or without ribavirin for treatment of hepatitis C virus genotype 1: a systematic review and meta-analysis. Clin Drug Investig. 2017;37(11):1009–23. https://doi.org/10.1007/s40261-017-0565-5.
56. Chayama K, Suzuki F, Karino Y, Kawakami Y, Sato K, Atarashi T, et al. Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 1 hepatitis C virus infection with and without cirrhosis. J Gastroenterol. 2018;53(4):557–65. https://doi.org/10.1007/s00535-017-1391-5.
57. Feld JJ, Jacobson IM, Hezode C, Asselah T, Ruane PJ, Gruener N, et al. Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infection. N Engl J Med. 2015;373(27):2599–607. https://doi.org/10.1056/NEJMoa1512610.
58. Foster GR, Afdhal N, Roberts SK, Brau N, Gane EJ, Pianko S, et al. Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. N Engl J Med. 2015;373(27):2608–17. https://doi.org/10.1056/NEJMoa1512612.
59. Klibanov OM, Gale SE, Santevecchi B. Ombitasvir/paritaprevir/ritonavir and dasabuvir tablets for hepatitis C virus genotype 1 infection. Ann Pharmacother. 2015;49(5):566–81. https://doi.org/10.1177/1060028015570729.
60. McCombs J, Matsuda T, Tonnu-Mihara I, Saab S, Hines P, L'Italien G, et al. The risk of long-term morbidity and mortality in patients with chronic hepatitis C: results from an analysis of data from a Department of Veterans Affairs Clinical Registry. JAMA Intern Med. 2014;174(2):204–12. https://doi.org/10.1001/jamainternmed.2013.12505.
61. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. Ann Intern Med. 2013;158(5 Pt 1):329–37. https://doi.org/10.7326/0003-4819-158-5-201303050-00005.
62. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. J Hepatol. 2017; https://doi.org/10.1016/j.jhep.2017.08.030.
63. Li DK, Chung RT. Impact of hepatitis C virus eradication on hepatocellular carcinogenesis. Cancer. 2015;121(17):2874–82. https://doi.org/10.1002/cncr.29528.
64. Hepatitis B. World Health Organization. 2017. http://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
65. Franco E, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: epidemiology and prevention in developing countries. World J Hepatol. 2012;4(3):74–80. https://doi.org/10.4254/wjh.v4.i3.74.
66. McMahon JB. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49(S5):S45–55. https://doi.org/10.1002/hep.22898.
67. Chih-Lin L, Jia-Horng K. Risk stratification for hepatitis B virus related hepatocellular carcinoma. J Gastroenterol Hepatol. 2013;28(1):10–7. https://doi.org/10.1111/jgh.12010.
68. Paganelli M, Stephenne X, Sokal EM. Chronic hepatitis B in children and adolescents. J Hepatol. 2012;57(4):885–96. https://doi.org/10.1016/j.jhep.2012.03.036.
69. Mittal S, El-Serag HB. Epidemiology of HCC: consider the population. J Clin Gastroenterol. 2013;47(Suppl):S2–6. https://doi.org/10.1097/MCG.0b013e3182872f29.

70. Do AL, Wong CR, Nguyen LH, Nguyen VG, Trinh H, Nguyen MH. Hepatocellular carcinoma incidence in noncirrhotic patients with chronic hepatitis B and patients with cirrhosis of all etiologies. J Clin Gastroenterol. 2014;48(7):644–9. https://doi.org/10.1097/mcg.0000000000000015.
71. Sung W-K, Zheng H, Li S, Chen R, Liu X, Li Y, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet. 2012;44:765. https://doi.org/10.1038/ng.2295; https://www.nature.com/articles/ng.2295#supplementary-information.
72. Jiang S, Yang Z, Li W, Li X, Wang Y, Zhang J, et al. Re-evaluation of the carcinogenic significance of hepatitis B virus integration in hepatocarcinogenesis. PLoS One. 2012;7(9):e40363. https://doi.org/10.1371/journal.pone.0040363.
73. Seeger C, Mason WS. Molecular biology of hepatitis B virus infection. Virology. 2015;479–480:672–86. https://doi.org/10.1016/j.virol.2015.02.031.
74. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350(11):1118–29. https://doi.org/10.1056/NEJMra031087.
75. Tian Y, Xiao X, Gong X, Peng F, Xu Y, Jiang Y, et al. HBx promotes cell proliferation by disturbing the cross-talk between miR-181a and PTEN. Sci Rep. 2017;7:40089. https://doi.org/10.1038/srep40089.
76. Dan Y, Zhang Y, Cheng L, Ma J, Xi Y, Yang L, et al. Hepatitis B virus X protein (HBx)-induced abnormalities of nucleic acid metabolism revealed by 1H-NMR-based metabonomics. Sci Rep. 2016;6:24430. https://doi.org/10.1038/srep24430.
77. Muroyama R, Kato N, Yoshida H, Otsuka M, Moriyama M, Wang Y, et al. Nucleotide change of codon 38 in the X gene of hepatitis B virus genotype C is associated with an increased risk of hepatocellular carcinoma. J Hepatol. 2006;45(6):805–12. https://doi.org/10.1016/j.jhep.2006.07.025.
78. Ringelhan M, O'Connor T, Protzer U, Heikenwalder M. The direct and indirect roles of HBV in liver cancer: prospective markers for HCC screening and potential therapeutic targets. J Pathol. 2015;235(2):355–67. https://doi.org/10.1002/path.4434.
79. Schillie S, Vellozzi C, Reingold A, Harris A, Haber P, Ward JW, et al. Prevention of hepatitis B virus infection in the United States: recommendations of the advisory committee on immunization practices. MMWR Recomm Rep. 2018;67(1):1–31. https://doi.org/10.15585/mmwr.rr6701a1.
80. Nayagam S, Thursz M, Sicuri E, Conteh L, Wiktor S, Low-Beer D, et al. Requirements for global elimination of hepatitis B: a modelling study. Lancet Infect Dis. 2016;16(12):1399–408. https://doi.org/10.1016/s1473-3099(16)30204-3.
81. Chang M-H, Chen C-J, Lai M-S, Hsu H-M, Wu T-C, Kong M-S, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N Engl J Med. 1997;336(26):1855–9. https://doi.org/10.1056/nejm199706263362602.
82. Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of hepatitis B virus infection and impact of vaccination on disease. Clin Liver Dis. 2016;20(4):607–28. https://doi.org/10.1016/j.cld.2016.06.006.
83. Childs L, Roesel S, Tohme RA. Status and progress of hepatitis B control through vaccination in the South-East Asia region, 1992-2015. Vaccine. 2018;36(1):6–14. https://doi.org/10.1016/j.vaccine.2017.11.027.
84. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57(1):167–85. https://doi.org/10.1016/j.jhep.2012.02.010.
85. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016;63(1):261–83. https://doi.org/10.1002/hep.28156.
86. Zhang Y-Q, Guo J-S. Antiviral therapies for hepatitis B virus-related hepatocellular carcinoma. World J Gastroenterol: WJG. 2015;21(13):3860–6. https://doi.org/10.3748/wjg.v21.i13.3860.
87. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295(1):65–73. https://doi.org/10.1001/jama.295.1.65.

88. Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. Hepatology. 2003;37(6):1309–19. https://doi.org/10.1053/jhep.2003.50208.
89. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology. 2009;49(5):1503–14. https://doi.org/10.1002/hep.22841.
90. Woo G, Tomlinson G, Nishikawa Y, Kowgier M, Sherman M, Wong DKH, et al. Tenofovir and entecavir are the most effective antiviral agents for chronic hepatitis B: a systematic review and Bayesian meta-analyses. Gastroenterology. 2010;139(4):1218–29.e5. https://doi.org/10.1053/j.gastro.2010.06.042.
91. Wang Y-C, Yang S-S, Su C-W, Wang Y-J, Lee K-C, Huo T-I, et al. Predictors of response to pegylated interferon in chronic hepatitis B: a real-world hospital-based analysis. Sci Rep. 2016;6:29605. https://doi.org/10.1038/srep29605; https://www.nature.com/articles/srep29605#supplementary-information.
92. Fung J, Lai C-L, Seto W-K, Yuen M-F. Nucleoside/nucleotide analogues in the treatment of chronic hepatitis B. J Antimicrob Chemother. 2011;66(12):2715–25. https://doi.org/10.1093/jac/dkr388.
93. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013;381(9865):468–75. https://doi.org/10.1016/S0140-6736(12)61425-1.
94. Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology. 2010;52(3):886–93. https://doi.org/10.1002/hep.23785.
95. Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology. 2006;44(6):1656–65. https://doi.org/10.1002/hep.21422.
96. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology. 2013;58(1):98–107. https://doi.org/10.1002/hep.26180.
97. WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: WHO; 2015.
98. Arends P, Sonneveld MJ, Zoutendijk R, Carey I, Brown A, Fasano M, et al. Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians. Gut. 2015;64(8):1289–95. https://doi.org/10.1136/gutjnl-2014-307023.
99. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, et al. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. Lancet Gastroenterol Hepatol. 2017;2(12):900–9. https://doi.org/10.1016/S2468-1253(17)30295-9.
100. Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384(9959):2053–63. https://doi.org/10.1016/S0140-6736(14)60220-8.
101. Zoulim F, Durantel D. Antiviral therapies and prospects for a cure of chronic hepatitis B. Cold Spring Harb Perspect Med. 2015;5(4):pii: a021501. https://doi.org/10.1101/cshperspect.a021501.
102. Rapti I, Hadziyannis S. Risk for hepatocellular carcinoma in the course of chronic hepatitis B virus infection and the protective effect of therapy with nucleos(t)ide analogues. World J Hepatol. 2015;7(8):1064–73. https://doi.org/10.4254/wjh.v7.i8.1064.
103. Study of ARB-001467 in subjects with chronic HBV infection receiving nucleos(t)ide analogue therapy. https://ClinicalTrials.gov/show/NCT02631096.
104. Study of ARO-HBV in normal adult volunteers and patients with hepatitis B virus (HBV). https://ClinicalTrials.gov/show/NCT03365947.

105. Blank A, Markert C, Hohmann N, Carls A, Mikus G, Lehr T, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. J Hepatol. 2016;65(3):483–9. https://doi.org/10.1016/j.jhep.2016.04.013.
106. A study of treatment with RO6864018 in virologically suppressed participants with chronic hepatitis B virus (HBV) infection. https://ClinicalTrials.gov/show/NCT02391805.
107. A study evaluating the safety, pharmacokinetics, and pharmacodynamics of RO6864018 in male, healthy, Asian participants. https://ClinicalTrials.gov/show/NCT02015715.
108. A study evaluating the safety, pharmacokinetics, and antiviral efficacy of SB 9200 in subjects infected with chronic HBV. https://ClinicalTrials.gov/show/NCT02751996.
109. Dose-ranging study to assess the safety, PK and initial antiviral efficacy of NVR 3–778 in chronic HBV patients. https://ClinicalTrials.gov/show/NCT02401737.
110. An efficacy, safety, and pharmacokinetics study of JNJ-56136379 in participants with chronic hepatitis B virus infection. https://ClinicalTrials.gov/show/NCT03361956.
111. A study of orally administered JNJ-56136379 to evaluate safety, tolerability and pharmacokinetics after single ascending doses and one multiple dose regimen in healthy participants (part I), and after multiple dose regimens in participants with chronic hepatitis B (part II). https://ClinicalTrials.gov/show/NCT02662712.
112. A study evaluating ABI-H0731 as adjunctive therapy in HBeAg-positive patients with chronic hepatitis B. https://ClinicalTrials.gov/show/NCT03576066.
113. Safety, tolerability, pharmacokinetics and antiviral activity of IONIS-HBVRx in treatment-naïve patients with chronic HBV infection. https://ClinicalTrials.gov/show/NCT02981602.
114. Testoni B, Durantel D, Zoulim F. Novel targets for hepatitis B virus therapy. Liver Int. 2017;37(Suppl 1):33–9. https://doi.org/10.1111/liv.13307.
115. Seeger C, Sohn JA. Targeting hepatitis B virus with CRISPR/Cas9. Mol Ther Nucleic Acids. 2014;3:e216. https://doi.org/10.1038/mtna.2014.68.
116. Kennedy EM, Kornepati AV, Cullen BR. Targeting hepatitis B virus cccDNA using CRISPR/Cas9. Antivir Res. 2015;123:188–92. https://doi.org/10.1016/j.antiviral.2015.10.004.
117. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science. 2014;343(6176):1221–8. https://doi.org/10.1126/science.1243462.
118. Decorsiere A, Mueller H, van Breugel PC, Abdul F, Gerossier L, Beran RK, et al. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature. 2016;531(7594):386–9. https://doi.org/10.1038/nature17170.
119. Testino G, Leone S, Borro P. Alcohol and hepatocellular carcinoma: a review and a point of view. World J Gastroenterol: WJG. 2014;20(43):15943–54. https://doi.org/10.3748/wjg.v20.i43.15943.
120. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology. 2004;127(5 Suppl 1):S87–96.
121. NIAAA. Alcohol facts and statistics. National Institute on Alcohol Abuse and Alcoholism. 2017. https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics.
122. Mann R. The epidemiology of alcoholic liver disease. National Institute on Alcohol Abuse and Alcoholism. 2004. https://pubs.niaaa.nih.gov/publications/arh27-3/209-219.htm.
123. NIDA. Sex and gender differences in substance use. National Institute on Drug Abuse. 2016. https://www.drugabuse.gov/publications/research-reports/substance-use-in-women/sex-gender-differences-in-substance-use.
124. Ramadori P, Cubero FJ, Liedtke C, Trautwein C, Nevzorova YA. Alcohol and hepatocellular carcinoma: adding fuel to the flame. Cancers. 2017;9(10):130. https://doi.org/10.3390/cancers9100130.
125. Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol (Fayetteville, NY). 2004;34(1):9–19. https://doi.org/10.1016/j.alcohol.2004.07.008.
126. Purohit V, Rapaka R, Kwon OS, Song BJ. Roles of alcohol and tobacco exposure in the development of hepatocellular carcinoma. Life Sci. 2013;92(1) https://doi.org/10.1016/j.lfs.2012.10.009.

127. Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology. 2002;36(5):1206–13. https://doi.org/10.1053/jhep.2002.36780.
128. Perlemuter G, Letteron P, Carnot F, Zavala F, Pessayre D, Nalpas B, et al. Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic mouse model. J Hepatol. 2003;39(6):1020–7.
129. Addolorato G, Mirijello A, Barrio P, Gual A. Treatment of alcohol use disorders in patients with alcoholic liver disease. J Hepatol. 2016;65(3):618–30. https://doi.org/10.1016/j.jhep.2016.04.029.
130. Petrick JL, Campbell PT, Koshiol J, Thistle JE, Andreotti G, Beane-Freeman LE, et al. Tobacco, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: the liver cancer pooling project. Br J Cancer. 2018;118(7):1005–12. https://doi.org/10.1038/s41416-018-0007-z.
131. Shih WL, Chang HC, Liaw YF, Lin SM, Lee SD, Chen PJ, et al. Influences of tobacco and alcohol use on hepatocellular carcinoma survival. Int J Cancer. 2012;131(11):2612–21. https://doi.org/10.1002/ijc.27508.
132. Heckley GA, Jarl J, Asamoah BO, G-Gerdtham U. How the risk of liver cancer changes after alcohol cessation: a review and meta-analysis of the current literature. BMC Cancer. 2011;11:446. https://doi.org/10.1186/1471-2407-11-446.
133. Fleming MF, Mundt MP, French MT, Manwell LB, Stauffacher EA, Barry KL. Brief physician advice for problem drinkers: long-term efficacy and benefit-cost analysis. Alcohol Clin Exp Res. 2002;26(1):36–43.
134. Moyer A, Finney JW, Swearingen CE, Vergun P. Brief interventions for alcohol problems: a meta-analytic review of controlled investigations in treatment-seeking and non-treatment-seeking populations. Addiction. 2002;97(3):279–92.
135. Kaner EF, Beyer F, Dickinson HO, Pienaar E, Campbell F, Schlesinger C, et al. Effectiveness of brief alcohol interventions in primary care populations. Cochrane Database Syst Rev. 2007;2:CD004148. https://doi.org/10.1002/14651858.CD004148.pub3.
136. Kaner EF, Beyer FR, Muirhead C, Campbell F, Pienaar ED, Bertholet N, et al. Effectiveness of brief alcohol interventions in primary care populations. Cochrane Database Syst Rev. 2018;(2):CD004148. https://doi.org/10.1002/14651858.CD004148.pub4.
137. Bendtsen P, Anderson P, Wojnar M, Newbury-Birch D, Mussener U, Colom J, et al. Professional's attitudes do not influence screening and brief interventions rates for hazardous and harmful drinkers: results from ODHIN study. Alcohol Alcohol. 2015;50(4):430–7. https://doi.org/10.1093/alcalc/agv020.
138. Owens L, Rose A, Thompson A, Pirmohamed M, Gilmore I, Richardson P. P1112: baclofen: maintenance of abstinence in alcohol dependent patients attending liver clinic. J Hepatol. 2015;62:S767. https://doi.org/10.1016/S0168-8278(15)31309-X.
139. Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonghia L, Mirijello A, et al. Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-blind controlled study. Lancet. 2007;370(9603):1915–22. https://doi.org/10.1016/S0140-6736(07)61814-5.
140. Wang T, Huang W, Chen F. Baclofen, a GABAB receptor agonist, inhibits human hepatocellular carcinoma cell growth in vitro and in vivo. Life Sci. 2008;82(9–10):536–41. https://doi.org/10.1016/j.lfs.2007.12.014.
141. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84. https://doi.org/10.1002/hep.28431.
142. Baffy G. Hepatocellular carcinoma in non-alcoholic fatty liver disease: epidemiology, pathogenesis, and prevention. J Clin Transl Hepatol. 2013;1(2):131–7. https://doi.org/10.14218/jcth.2013.00005.
143. Ong JP, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. Clin Liver Dis. 2007;11(1):1–16, vii. https://doi.org/10.1016/j.cld.2007.02.009.
144. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol. 2012;56(6):1384–91. https://doi.org/10.1016/j.jhep.2011.10.027.

145. Janus O, Zobair MY, Vishnu R, Lyn PL, Terry G, James M, et al. Cryptogenic cirrhosis and posttransplantation nonalcoholic fatty liver disease. Liver Transpl. 2001;7(9):797–801. https://doi.org/10.1053/jlts.2001.24644.
146. Eduardo F, Estela Á, Nora D, Graciela L, Cristina L. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology. 2004;40(4):820–6. https://doi.org/10.1002/hep.1840400411.
147. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129(1):113–21.
148. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. Clin Gastroenterol Hepatol. 2012;10(12):1342–59.e2. https://doi.org/10.1016/j.cgh.2012.10.001.
149. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology. 2002;123(1):134–40.
150. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444:860. https://doi.org/10.1038/nature05485.
151. Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, et al. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res. 2007;67(6):2497–507. https://doi.org/10.1158/0008-5472.Can-06-3075.
152. Sara A, Ilaria P, Cristiana B, Maurizio P, Sebastiano C, Erica N, et al. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. Hepatology. 2005;42(6):1339–48. https://doi.org/10.1002/hep.20965.
153. Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. Endocr Rev. 2012;33(4):547–94. https://doi.org/10.1210/er.2011-1015.
154. Alexia C, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A. 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. 2004;68(6):1003–15. https://doi.org/10.1016/j.bcp.2004.05.029.
155. Che L, Pilo MG, Cigliano A, Latte G, Simile MM, Ribback S, et al. Oncogene dependent requirement of fatty acid synthase in hepatocellular carcinoma. Cell Cycle. 2017;16(6):499–507. https://doi.org/10.1080/15384101.2017.1282586.
156. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7(10):763–77. https://doi.org/10.1038/nrc2222.
157. Yamashita T, Honda M, Takatori H, Nishino R, Minato H, Takamura H, et al. Activation of lipogenic pathway correlates with cell proliferation and poor prognosis in hepatocellular carcinoma. J Hepatol. 2009;50(1):100–10. https://doi.org/10.1016/j.jhep.2008.07.036.
158. Bugianesi E. Non-alcoholic steatohepatitis and cancer. Clin Liver Dis. 2007;11(1):191–207, x–xi. https://doi.org/10.1016/j.cld.2007.02.006.
159. Yang S, Zhu H, Li Y, Lin H, Gabrielson K, Trush MA, et al. Mitochondrial adaptations to obesity-related oxidant stress. Arch Biochem Biophys. 2000;378(2):259–68. https://doi.org/10.1006/abbi.2000.1829.
160. Hu W, Feng Z, Eveleigh J, Iyer G, Pan J, Amin S, et al. The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. Carcinogenesis. 2002;23(11):1781–9.
161. Xu Z, Chen L, Leung L, Yen TS, Lee C, Chan JY. Liver-specific inactivation of the Nrf1 gene in adult mouse leads to nonalcoholic steatohepatitis and hepatic neoplasia. Proc Natl Acad Sci U S A. 2005;102(11):4120–5. https://doi.org/10.1073/pnas.0500660102.
162. Stickel F, Hellerbrand C. Non-alcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: mechanisms and implications. Gut. 2010;59(10):1303–7. https://doi.org/10.1136/gut.2009.199661.

163. Fabio M, Cristiana B. Adipokines in liver diseases. Hepatology. 2009;50(3):957–69. https://doi.org/10.1002/hep.23046.
164. He G, Karin M. NF-kappaB and STAT3—key players in liver inflammation and cancer. Cell Res. 2011;21(1):159–68. https://doi.org/10.1038/cr.2010.183.
165. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology. 2010;51(5):1820–32. https://doi.org/10.1002/hep.23594.
166. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology. 2015;149(2):367–78.e5. https://doi.org/10.1053/j.gastro.2015.04.005.
167. Vilar-Gomez E, Calzadilla-Bertot L, Friedman SL, Gra-Oramas B, Gonzalez-Fabian L, Villa-Jimenez O, et al. Improvement in liver histology due to lifestyle modification is independently associated with improved kidney function in patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2017;45(2):332–44. https://doi.org/10.1111/apt.13860.
168. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–57. https://doi.org/10.1002/hep.29367.
169. Sung KC, Ryu S, Lee JY, Kim JY, Wild SH, Byrne CD. Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. J Hepatol. 2016;65(4):791–7. https://doi.org/10.1016/j.jhep.2016.05.026.
170. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2008;6(12):1396–402. https://doi.org/10.1016/j.cgh.2008.08.012.
171. Lassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. Gastroenterology. 2015;149(2):379–88; quiz e15–6. https://doi.org/10.1053/j.gastro.2015.04.014.
172. Mathurin P, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, et al. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. Gastroenterology. 2009;137(2):532–40. https://doi.org/10.1053/j.gastro.2009.04.052.
173. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med. 2012;366(17):1577–85. https://doi.org/10.1056/NEJMoa1200111.
174. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, et al. Bariatric surgery versus intensive medical therapy for diabetes—3-year outcomes. N Engl J Med. 2014;370(21):2002–13. https://doi.org/10.1056/NEJMoa1401329.
175. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med. 2012;366(17):1567–76. https://doi.org/10.1056/NEJMoa1200225.
176. Chavez-Tapia NC, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. Cochrane Database Syst Rev. 2010;(1):CD007340. https://doi.org/10.1002/14651858.CD007340.pub2.
177. Kral JG, Thung SN, Biron S, Hould FS, Lebel S, Marceau S, et al. Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. Surgery. 2004;135(1):48–58. https://doi.org/10.1016/j.surg.2003.10.003.
178. Klein S, Mittendorfer B, Eagon JC, Patterson B, Grant L, Feirt N, et al. Gastric bypass surgery improves metabolic and hepatic abnormalities associated with nonalcoholic fatty liver disease. Gastroenterology. 2006;130(6):1564–72. https://doi.org/10.1053/j.gastro.2006.01.042.
179. Dallal RM, Mattar SG, Lord JL, Watson AR, Cottam DR, Eid GM, et al. Results of laparoscopic gastric bypass in patients with cirrhosis. Obes Surg. 2004;14(1):47–53. https://doi.org/10.1381/096089204772787284.

180. Shimizu H, Phuong V, Maia M, Kroh M, Chand B, Schauer PR, et al. Bariatric surgery in patients with liver cirrhosis. Surg Obes Relat Dis. 2013;9(1):1–6. https://doi.org/10.1016/j.soard.2012.07.021.
181. Jan A, Narwaria M, Mahawar KK. A systematic review of bariatric surgery in patients with liver cirrhosis. Obes Surg. 2015;25(8):1518–26. https://doi.org/10.1007/s11695-015-1727-2.
182. Mosko JD, Nguyen GC. Increased perioperative mortality following bariatric surgery among patients with cirrhosis. Clin Gastroenterol Hepatol. 2011;9(10):897–901. https://doi.org/10.1016/j.cgh.2011.07.007.
183. Rizvon MK, Chou CL. Surgery in the patient with liver disease. Med Clin North Am. 2003;87(1):211–27.
184. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. Am J Gastroenterol. 2013;108:881. https://doi.org/10.1038/ajg.2013.5.
185. Zhou Y-Y, Zhu G-Q, Liu T, Zheng J-N, Cheng Z, Zou T-T, et al. Systematic review with network meta-analysis: antidiabetic medication and risk of hepatocellular carcinoma. Sci Rep. 2016;6:33743. https://doi.org/10.1038/srep33743.
186. Home PD, Kahn SE, Jones NP, Noronha D, Beck-Nielsen H, Viberti G. Experience of malignancies with oral glucose-lowering drugs in the randomised controlled ADOPT (A Diabetes Outcome Progression Trial) and RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) clinical trials. Diabetologia. 2010;53(9):1838–45. https://doi.org/10.1007/s00125-010-1804-y.
187. Promrat K, Lutchman G, Uwaifo GI, Freedman RJ, Soza A, Heller T, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology. 2004;39(1):188–96. https://doi.org/10.1002/hep.20012.
188. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355(22):2297–307. https://doi.org/10.1056/NEJMoa060326.
189. Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology. 2008;135(4):1176–84. https://doi.org/10.1053/j.gastro.2008.06.047.
190. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362(18):1675–85. https://doi.org/10.1056/NEJMoa0907929.
191. Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. Ann Intern Med. 2016;165(5):305–15. https://doi.org/10.7326/m15-1774.
192. Lago RM, Singh PP, Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. Lancet. 2007;370(9593):1129–36. https://doi.org/10.1016/s0140-6736(07)61514-1.
193. Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. JAMA. 2007;298(10):1180–8. https://doi.org/10.1001/jama.298.10.1180.
194. Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. Hepatology. 2007;46(2):424–9. https://doi.org/10.1002/hep.21661.
195. Demierre M-F, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer. 2005;5:930. https://doi.org/10.1038/nrc1751.
196. Mullen PJ, Yu R, Longo J, Archer MC, Penn LZ. The interplay between cell signalling and the mevalonate pathway in cancer. Nat Rev Cancer. 2016;16:718. https://doi.org/10.1038/nrc.2016.76.
197. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. Gastroenterology. 2013;144(2):323–32. https://doi.org/10.1053/j.gastro.2012.10.005.

198. Kim G, Jang SY, Nam CM, Kang ES. Statin use and the risk of hepatocellular carcinoma in patients at high risk: a nationwide nested case-control study. J Hepatol. 2018;68(3):476–84. https://doi.org/10.1016/j.jhep.2017.10.018.
199. Cholesterol Treatment Trialists Collaboration, Emberson JR, Kearney PM, Blackwell L, Newman C, Reith C, et al. Lack of effect of lowering LDL cholesterol on cancer: meta-analysis of individual data from 175,000 people in 27 randomised trials of statin therapy. PLoS One. 2012;7(1):e29849. https://doi.org/10.1371/journal.pone.0029849.
200. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2015;385(9972):956–65. https://doi.org/10.1016/S0140-6736(14)61933-4.
201. Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology. 2013;145(3):574–82.e1. https://doi.org/10.1053/j.gastro.2013.05.042.
202. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and -δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology. 2016;150(5):1147–59.e5.
203. Zimmermann HW, Seidler S, Nattermann J, Gassler N, Hellerbrand C, Zernecke A, et al. Functional contribution of elevated circulating and hepatic non-classical CD14CD16 monocytes to inflammation and human liver fibrosis. PLoS One. 2010;5(6):e11049. https://doi.org/10.1371/journal.pone.0011049.
204. Kazankov K, Barrera F, Moller HJ, Rosso C, Bugianesi E, David E, et al. The macrophage activation marker sCD163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. Liver Int. 2016;36(10):1549–57. https://doi.org/10.1111/liv.13150.
205. Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, et al. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology. 2018;67(5):1754–67. https://doi.org/10.1002/hep.29477.
206. Brenner C, Galluzzi L, Kepp O, Kroemer G. Decoding cell death signals in liver inflammation. J Hepatol. 2013;59(3):583–94. https://doi.org/10.1016/j.jhep.2013.03.033.
207. Budas G, Karnik S, Jonnson T, Shafizadeh T, Watkins S, Breckenridge D, et al. Reduction of liver steatosis and fibrosis with an ASK1 inhibitor in a murine model of nash is accompanied by improvements in cholesterol, bile acid and lipid metabolism. J Hepatol. 2016;64(2):S170. https://doi.org/10.1016/S0168-8278(16)01686-X.
208. Sumida Y, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. J Gastroenterol. 2018;53(3):362–76. https://doi.org/10.1007/s00535-017-1415-1.
209. Loomba R, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, et al. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. Hepatology (Baltimore, Md). 2018;67(2):549–59. https://doi.org/10.1002/hep.29514.
210. Komuves LG, Feren A, Jones AL, Fodor E. Expression of epidermal growth factor and its receptor in cirrhotic liver disease. J Histochem Cytochem. 2000;48(6):821–30. https://doi.org/10.1177/002215540004800610.
211. Fuchs BC, Hoshida Y, Fujii T, Wei L, Yamada S, Lauwers GY, et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology. 2014;59(4):1577–90. https://doi.org/10.1002/hep.26898.
212. Schiffer E, Housset C, Cacheux W, Wendum D, Desbois-Mouthon C, Rey C, et al. Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. Hepatology. 2005;41(2):307–14. https://doi.org/10.1002/hep.20538.
213. Tonjes RR, Lohler J, O'Sullivan JF, Kay GF, Schmidt GH, Dalemans W, et al. Autocrine mitogen IgEGF cooperates with c-myc or with the Hcs locus during hepatocarcinogenesis in transgenic mice. Oncogene. 1995;10(4):765–8.
214. Tanabe KK, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA. 2008;299(1):53–60. https://doi.org/10.1001/jama.2007.65.

215. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med. 2008;359(19):1995–2004. https://doi.org/10.1056/NEJMoa0804525.
216. Hoshida Y, Villanueva A, Sangiovanni A, Sole M, Hur C, Andersson KL, et al. Prognostic gene expression signature for patients with hepatitis C-related early-stage cirrhosis. Gastroenterology. 2013;144(5):1024–30. https://doi.org/10.1053/j.gastro.2013.01.021.
217. Jiang G, Yu K, Shao L, Yu X, Hu C, Qian P, et al. Association between epidermal growth factor gene +61A/G polymorphism and the risk of hepatocellular carcinoma: a meta-analysis based on 16 studies. BMC Cancer. 2015;15:314. https://doi.org/10.1186/s12885-015-1318-6.
218. Lanaya H, Natarajan A, Komposch K, Li L, Amberg N, Chen L, et al. EGFR has a tumour-promoting role in liver macrophages during hepatocellular carcinoma formation. Nat Cell Biol. 2014;16(10):972–7. https://doi.org/10.1038/ncb3031.
219. Simon TG, Ma Y, Ludvigsson JF, Chong DQ, Giovannucci EL, Fuchs CS, et al. Association between aspirin use and risk of hepatocellular carcinoma. JAMA Oncol. 2018;4(12):1683–90.
220. Malehmir M, et al. Platelet GPIbα is a mediator and potential interventional target for NASH and subsequent liver cancer. Nat Med. 2019;25(4):641–55.
221. Hanazaki K, Kajikawa S, Shimozawa N, Mihara M, Shimada K, Hiraguri M, et al. Survival and recurrence after hepatic resection of 386 consecutive patients with hepatocellular carcinoma. J Am Coll Surg. 2000;191(4):381–8.
222. Zimmerman MA, Ghobrial R, Tong MJ, et al. Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. Arch Surg. 2008;143(2):182–8. https://doi.org/10.1001/archsurg.2007.39.
223. Haruhiko Y, Yasushi S, Masatoshi K, Shuichiro S, Toshihiko M, Masamichi K, et al. Effect of vitamin K2 on the recurrence of hepatocellular carcinoma. Hepatology. 2011;54(2):532–40. https://doi.org/10.1002/hep.24430.
224. Okita K, Izumi N, Matsui O, Tanaka K, Kaneko S, Moriwaki H, et al. Peretinoin after curative therapy of hepatitis C-related hepatocellular carcinoma: a randomized double-blind placebo-controlled study. J Gastroenterol. 2015;50(2):191–202. https://doi.org/10.1007/s00535-014-0956-9.
225. Lu L-C, Cheng A-L, Poon RTP. Recent advances in the prevention of hepatocellular carcinoma recurrence. Semin Liver Dis. 2014;34(04):427–34. https://doi.org/10.1055/s-0034-1394141.
226. Zhuang L, Zeng X, Yang Z, Meng Z. Effect and safety of interferon for hepatocellular carcinoma: a systematic review and meta-analysis. PLoS One. 2013;8(9):e61361. https://doi.org/10.1371/journal.pone.0061361.
227. Lohitesh K, Chowdhury R, Mukherjee S. Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight. Cancer Cell Int. 2018;18:44. https://doi.org/10.1186/s12935-018-0538-7.
228. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64(19):7099–109. https://doi.org/10.1158/0008-5472.Can-04-1443.
229. Bruix J, Takayama T, Mazzaferro V, Chau G-Y, Yang J, Kudo M, et al. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2015;16(13):1344–54. https://doi.org/10.1016/S1470-2045(15)00198-9.
230. Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology. 1998;27(2):407–14. https://doi.org/10.1002/hep.510270214.
231. Flecken T, Schmidt N, Hild S, Gostick E, Drognitz O, Zeiser R, et al. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. Hepatology. 2014;59(4):1415–26. https://doi.org/10.1002/hep.26731.
232. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative,

phase 1/2 dose escalation and expansion trial. Lancet. 2017;389(10088):2492–502. https://doi.org/10.1016/S0140-6736(17)31046-2.
233. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018;19(7):940–52. https://doi.org/10.1016/S1470-2045(18)30351-6.
234. Takayama T, Makuuchi M, Sekine T, Terui S, Shiraiwa H, Kosuge T, et al. Distribution and therapeutic effect of intraarterially transferred tumor-infiltrating lymphocytes in hepatic malignancies. A preliminary report. Cancer. 1991;68(11):2391–6.
235. Takayama T, Sekine T, Kondo Y, Kakizoe T, Makuuchi M. Adjuvant adoptive immunotherapy against hepatocellular carcinoma. Hepatology. 1998;28(5):1436–7. https://doi.org/10.1002/hep.510280539.
236. Konomi Y, Sekine T, Takayama T, Fuji M, Tanaka T. Cytotoxic activity of CD4+ T cells against autologous tumor cells. Jpn J Cancer Res. 1995;86(9):854–60.
237. Lee JH, Lee J-H, Lim Y-S, Yeon JE, Song T-J, Yu SJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology. 2015;148(7):1383–91.e6. https://doi.org/10.1053/j.gastro.2015.02.055.
238. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, Yu S, et al. GS-008 – sustained efficacy of adjuvant immunotherapy with cytokineinduced killer cells for hepatocellular carcinoma: an extended 5-year follow-up. J Hepatol. 2018;68:S37–S8. https://doi.org/10.1016/S0168-8278(18)30291-5.
239. Xie Y, Xiang Y, Sheng J, Zhang D, Yao X, Yang Y, et al. Immunotherapy for hepatocellular carcinoma: current advances and future expectations. J Immunol Res. 2018;2018:8. https://doi.org/10.1155/2018/8740976.

Part IV
Molecular Pathogenesis and Therapeutic Discovery

Chapter 14
Molecular Alterations and Heterogeneity in Hepatocellular Carcinoma

Man Hsin Hung and Xin Wei Wang

Introduction

Hepatocellular carcinoma (HCC) is the most common form of primary hepatic tumors and is the second leading cause of global cancer-related death, responsible for more than 745,000 deaths every year [1]. Late diagnosis, high postoperative recurrence rate, and lack of effective treatment for patients with advanced disease explain the poor outcomes for most HCC patients. Identifying effective treatment for HCC has been a major research focus for decades as evidenced by the inclusion of more than 1200 clinical trials testing different interventions in the ClinitalTrials.gov database [2]. However, a majority of the above-listed clinical trials failed, and only sorafenib, regorafenib, and lenvatinib succeeded to show survival benefit in HCC, which subsequently received regulatory approval [3–5]. However, these treatments only provide a marginal improvement of overall survival with palliative intent. Furthermore, regardless of the final results of clinical trial, treatment response between patients varied, indicating the heterogeneous characteristic of HCC.

"Heterogeneity" means a state that consists of dissimilar or diverse elements [6]. Accordingly, heterogeneity of HCC means that patients given with an identical

M. H. Hung
Laboratory of Human Carcinogenesis, Bethesda, MD, USA

Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan

School of Medicine, National Yang-Ming University, Taipei, Taiwan

X. W. Wang (✉)
Laboratory of Human Carcinogenesis, Bethesda, MD, USA

Liver Cancer Program, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA
e-mail: xw3u@nih.gov

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_14

HCC diagnosis could be different. The concept describing HCC as a heterogeneous disease does not come out just in recent years; back in 1954, Edmondson introduced the well-known Edmondson-Steiner Grading system, which described that patients could be grouped by the grade of tumor differentiation, and tumors with worse differentiation were more likely to develop metastasis [7]. Using the same grading system, Kenmochi et al. demonstrated that 47.7% HCC cases had two or more areas within a tumor presented with different grade of differentiation [8]. The abovementioned studies reveal two different entities of heterogeneity; Edmondson's work pointed out diversity among patients with the same histological type of tumor, termed as "intertumoral heterogeneity" (Fig. 14.1), while results from Kenmochi et al. demonstrated the other entity – "intratumoral heterogeneity" – referring to heterogeneity among cancer cells within a single patient.

With the advent of molecular medicine, we know that the heterogeneity of HCC is not restricted in pathophenotypic differences but is linked to biological mechanisms driving tumor progression. In this chapter, we will start by establishing a general understanding of molecular heterogeneity linking to hepatocarcinogenesis. We will then explore the evidence of intertumoral and intratumoral molecular heterogeneity in HCC. Lastly, we will discuss the importance to integrate the knowledge of heterogeneity into tailor treatment for individual patients and potential challenges and opportunities ahead.

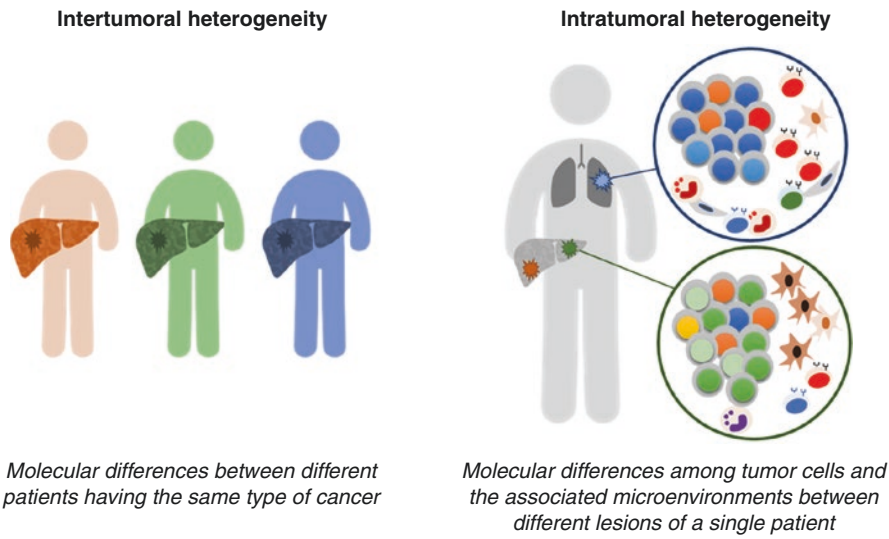

Fig. 14.1 Tumor heterogeneity of HCC. This cartoon summarized the concepts distinguishing intertumoral heterogeneity and intratumoral heterogeneity in HCC. Intertumoral heterogeneity denotes the variations among different HCC patients, and intratumoral heterogeneity refers to the differences among individual lesions and/or cells in a single patient

Molecular Alterations of HCC

Cancer is considered as a genetic disease, meaning that mutation(s) of genetic material lead to the initiation and progression of cancer [9]. According to the way by which a cancer cell obtains an alteration, genetic variances can be separated into two entities – germline mutation and somatic mutation. Germline mutation are defined as genetic variances that develop within the heritable genome and are transmitted from parent to offspring, whereas somatic mutations, which account for most genetic aberrations found in cancer, are acquired de novo by cancer cells [10]. Up to 90% of genetic variances in tumors are somatic mutations, which arise from replication errors or from DNA damage with incorrect repair [11]. Common factors triggering the development of somatic mutation in HCC could be divided into two categories: the exogenous mutagens include oxidative and hypoxia stress plus exposure of chemicals or radiation such as UV, while endogenous mutagens include defective DNA repair machinery or other factors related to genomic stability. Notably, among all the mutations identified, only a fraction of mutations, termed driver mutations, can confer a survival advantage to cancer cells, leading to preferential growth, survival, and metastasis.

In the following section, we will review data related to different types of molecular alterations and learn how they affect the biology of HCC.

Genomic Alteration

In HCC, several different kinds of genomic alterations have been reported and are summarized as follows: change of gene copy numbers, chromosomal rearrangement, mutation, and viral genome insertion [12].

Copy Number Variation

Copy number variation (CNV) refers to a DNA segment of one kilobase or larger with variable copy number (could be number gain, loss, or amplification) as compared to a reference genome [13]. The presence of CNV may change the physical arrangement of genes on chromosomes, leading to functional alterations of involved genes. Compared to healthy populations, CNVs are more frequently found in patients with cancer, and they are more likely to occur in regions containing cancer driver genes and/or tumor suppressor genes [14, 15]. In HCC, CNVs had been found to affect many important oncogenes, such as *MYC, MET*, *MDM4*, and *YY1AP1*, and tumor suppressor genes, like *PTEN* and *RB1* [12, 15–19]. Totoki et al. used a bioinformatic algorism to assess CNVs and showed that recurrent focal amplification was more frequently observed than homozygous deletion, and a fraction of patients (28.9% in his cohort) presented with concurrent high CNVs and ploidy change, suggesting a high degree of structural changes in the whole genome [20].

Somatic Mutation

With the increasing use of next-generation sequencing, researchers are able to explore the human genome in more depth and advance our knowledge of cancer biology. There are two major platforms, whole-exome sequencing and whole-genome sequencing, being widely adapted for large-scale DNA sequencing studies today. Whole-exome sequencing captures and sequences the DNA fragments containing exonic regions, which enable the comprehensive detection of somatic alterations in the protein-coding regions and has led to the discovery of many novel genes implicated in carcinogenesis. However, whole-exome sequencing covers only about 1% of human genome and may miss important information in the noncoding regions. In contrast, whole-genome sequencing provides a full coverage of human genome, allowing identification of all genetic events, such as substitutions, structural rearrangement, and viral genome integrations, that may occur in coding and noncoding regions. Both methods have been widely adapted for HCC genomic studies, providing valuable information about the genetic mechanism of hepatocarcinogenesis.

Nucleotide Substitution Signature

There are six patterns of somatic base substitutions, namely, C > A/G > T, C > G/G > C, C > T/G > A, T > A/A > T, T > C/A > G, and T > G/A > C, which could occur and cause a point mutation of a gene. Notably, the choice of substitution is not made by random selection; several studies suggested that the patterns of substitutions could be indicative of a specific mutagenesis mechanism occurring in tumor cells. Totoki et al. reported the first whole sequencing study and showed that C > T/G > A and T > C/A > G substitutions are dominant in a HCV-related HCC patient [21]. Similar substitution patterns were shown in larger cohorts and in patients with hepatitis B virus (HBV)-related HCC [22, 23]. Interestingly, the substitution patterns change in patients with HCC related to nonviral etiologies; A > T/T > A transversions at [C|T]AG trinucleotide motifs were associated with aristolochic acid (a plant-derived carcinogen), and G > T/C > A was highly correlated with aflatoxin B1 exposure, which may lead to TP53 249S mutations [24–26], though Guichard et al. showed that G > T/C > A substitution was also enriched in well-differentiated tumors and tumors that developed on non-cirrhotic livers [27].

Significant Mutated Genes

Based on the original idea of tumor development, mutational activation of oncogene and loss-of-function mutation of tumor suppressor gene lead to cancer initiation and progression. Therefore, genes with higher degrees of mutation are more likely to be critical in cancer biology.

Genome-wide sequencing studies provided landscape views of genetic alterations and identified recurrent mutated genes in HCC [22–24, 27, 28]. Several genes, such as *TERT* promoter, *TP53*, and *CTNNB*, were commonly mutated in different cohort, suggesting that these genes may be functionally critical in HCC.

HBV Genome Integration

Chronic HBV infection is an important etiology associated with the development of HCC, particularly in China and other HBV endemic areas [29]. HBV is a DNA virus, and integration of a viral DNA into the host genome is one of the mechanisms by which HBV promotes hepatocarcinogenesis [30]. Around 85% of HBV-infected HCC patients exhibited evidence of HBV DNA integration in the host genome, and interestingly, the occurrence of integration is more enriched in the tumor part than in the adjacent normal liver [24, 30]. The HBV integration breakpoints can be found across the whole genome, and approximately 50% of them occur within several particular genes, such as *TERT* and *MLL4* [22–24, 30]. Insertion of HBV DNA results in change of gene expression (mostly upregulation) and may alter chromosomal stability and trigger CNVs [22, 30].

Epigenomic Alteration

Epigenetic alterations refer to the molecular changes, such as DNA methylation, chromatin remodeling, and noncoding RNAs, that affect gene function independent of changing the DNA sequence of a gene [31]. Epigenetic changes are highly prevalent in many different types of cancer, including HCC. Several studies had addressed the importance of epigenetic regulation in affecting hepatocarcinogenesis. Genome-wide DNA methylation profiles showed that a tumor exerted a significant increment in both hypo- and hypermethylation in comparison to a paired non-tumor, and tumor-specific hypermethylation determined the expressions of *CDKN2A*, *HHIP*, *PTGR1*, *TMEM106A*, *MT1M*, *MT1E*, and *CPS1* [24]. On the other hand, emerging evidence showed that dysregulation of microRNAs, a class of short, noncoding RNA, contributed to activation of oncogenic signaling in HCC; for instance, Meng et al. showed that overexpression of miR-21 inhibits the expression of the phosphatase and tensin homolog (PTEN) tumor suppressor [32], and Coulourn et al. described that loss of miR-122 expression in liver tumor significantly enhances metastatic properties of cancer cell through upregulation of a network involving VEGF, HIF1A, RAC1, RHOA, and vimentin [33]. Lastly, studies also showed that genes related to chromatin modification were frequently altered in HCC [20, 24].

Key Driving Genes and Pathways in HCC

In recent decades, comprehensive studies on liver cancer genome had identified many recurrently mutated genes in HCC and improve our understanding of hepatocarcinogenesis. By exploring aggregation of altered genes, important oncogenic pathways in HCC were recognized, which provided a more functional understanding regarding the development and progression of HCC. Here, we will introduce the most frequently altered pathway in HCC.

TP53 Pathway

Somatic mutations in the *TP53* gene are the most frequently altered events in human cancer [34]. In HCC, mutations in TP53, mostly inactivation mutation, could be found in 18–37% of patients [20, 24, 30]. Notably, some patients exert a mutation-independent p53 inactivation mechanism [35]; 23% of patients in the TCGA cohort exhibited downregulation of p53 target genes (surrogate for p53 inactivation) without detectable TP53 mutations [24]. Furthermore, tumors with low p53 activity inferred by the p53 target signature were associated with increased copy number instability, higher pathological grade, reduced expression of mature hepatocyte signature, and increased risk of tumor recurrence [24]. On the other hand, alterations of several other genes within the TP53 network, namely, *IRF2*, *MDM2/MDM4*, *ATM*, *RPS6KA3*, *CDKN2A*, *RB1*, *CDK4*, *CCND1*, and *CCNE*, were also identified, resulting in a high prevalence (up to 72%) of TP53 signaling alterations in HCC [20, 24].

Wnt/β-Catenin Pathway

Aberrant activation of Wnt signaling is a critical molecular event driving hepatocarcinogenesis. There are several different genes involving this signaling pathway. Somatically acquired missense mutation in exon 3 of the *CTNNB1* (β-catenin) gene, which leads to constant activation of β-catenin by preventing phosphorylation of β-catenin, is the most common molecular change related to this signaling pathway (frequency ranging from 10% to 32.8% in genome-wide sequencing studies) [12, 30]. Other alterations, such as epigenetic inactivation of *SFRPs* and *SOX*, inactivating mutation of *AXIN1* or *APC*, and upregulation of *FGF19*, *MYC*, and *CCND1*, were also reported in HCC cohorts [20, 24]. Collectively, alterations of the Wnt-associated signaling pathway are observed in 44–66% of patients with HCC.

TERT Pathway

To obtain the ability of infinite replication, activation of telomerase (encoded by *TERT* gene) is required for cancer cells [12]. Tokoki et al. reported that 54% of HCC patients in their cohort had somatic mutation of *TERT* gene at its promoter region,

and the percentage of *TERT* mutation was higher in HCV-positive cases (64%) in comparison to nonviral (59%) and HBV-positive cases (37%) [20]. Similar results were shown in the TCGA cohort; *TERT* mutation was found in 44% of patients and enriched in HCV-related HCC patients [24]. Additionally, the occurrence of TERT promoter mutation was frequently found with *CDKN2A* silencing, which further enhanced the expression of *TERT* through downregulation of $p16^{INK4A}$ [24, 36].

Chromatin Remodeling Pathway

Chromatin refers to the DNA-protein complex within the nucleus that helps to package DNA into a more compact and denser structure [37]. DNA interacts with histone protein via covalent bonding and forms a nucleosome, the basic structure of chromatin. Thus, modification of histone protein, including acetylation or methylation, would affect the DNA-histone structure (e.g., open or closed), leading to change of gene expression [12].

In HCC, alterations of *ARID1A*, *ARID1B*, and *ARID2* are frequently observed in HCC patients [20, 24]. The ARID family genes encode the core proteins of a nucleosome remodeling complex, SWI/SNF (switch/sucrose non-fermentable); alterations of these genes, such as frameshift mutations, copy number loss, and homozygous deletion, lead to dysregulation of chromatin [38]. In addition to ARID family, mutations *of BAP1*, *KMT2D CREBBP* were reported in HCC patients. In sum, alterations of chromatin modifier genes could be identified in nearly 50% of HCC patients.

PI3K-mTOR Pathway

The phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway is critical to cell growth and angiogenesis; *MET*, *FGFR*, *VEGFA*, and several other growth factors can activate *PI3K*/*AKT* and *mTOR* signaling, while PTEN counteracts with activation of this signaling pathway [39]. Aberrant activation of PI3K-related pathway is a common driving mechanism in many types of cancers. In HCC, oncogenic activation of PI3K- mTOR signaling affects about 45% of patients, and inactivating mutation of *TSC1-TSC2* is the most common contributing factor [20].

Nrf2-KEAP1 Pathway

Nuclear factor-like 2 (encoded by *NFE2L2*, also known as NRF2) is a transcriptional factor that regulates many genes associated with antioxidation and metabolism [40]. NRF2 is regulated by KEAP1 via the ubiquitin-proteasome pathway; activation of the missense mutation of *NFE2L2* and inactivation of the mutation of the *KEAP1* gene are recurrently seen in HCC [12].

Metabolic Alteration and Metabolomic Investigation

Metabolic reprogramming, which describes serial changes involving nutrition uptake and utilization, can fuel cancer cell growth and proliferation [41]. In HCC, accelerated glucose uptake and preferential activation of pyruvate kinase muscle isozyme M2 (PKM2)-mediated glycolysis had been shown to increase the proliferation and progression of liver cancer cell [42]. Also, dysregulated glutamine metabolism and increased de novo lipogenesis were also found to promote the development of therapeutic resistance and cell survival in HCC [43, 44].

Metabolomics is the global and unbiased survey of the complement of small molecules (<1 kDa) in a biological sample (could be biofluid, tissue, organ, or organism) and measures the end products of various metabolic processes happening in cells [45]. Several studies had applied this method and successfully identified specific metabolic profiles that help early diagnosis and clinical outcome prediction in HCC [46–48].

Intertumoral Heterogeneity in HCC

Intertumoral heterogeneity refers to the diversity between different HCC patients. Many different factors contributing to the generation of intertumoral heterogeneity in HCC have been identified and could be summarized into three major categories: environmental exposures, individual genetic predispositions, and somatic molecular alterations [26, 29, 46, 47, 49, 50].

Environmental Exposure

The development of HCC is known to be associated with chronic liver inflammation induced by various different exposures, such as hepatitis viruses, alcohol, smoking, and aflatoxin exposure [29]. Therefore, recognizing different environmental exposures is the first step in characterizing the heterogeneity of HCC among different patients.

Nearly 80% of HCC is contributed by hepatitis B virus (HBV) or hepatitis C virus (HCV) infections [51]. Though liver tumors related to chronic HBV and HCV infection are histologically similar, each virus has very distinct mechanisms driving hepatocarcinogenesis. Several large-scale genomic studies showed that liver tumors associated with different viruses had distinct mutation patterns [23, 24, 28]. HBV is a DNA virus meaning that a viral genome would integrate into the host genome and may, therefore, affect the integrity of the host genome and alter gene expression near the integration site [52]. HBV-infected tumors were characterized by increasing frequency of *TP53* mutation and hyperexpression of *CCND1*,

CCNE1, *GLI2*, *TERT*, and *MLL4*, which seem to be associated with viral DNA integration into the host genome [23, 24, 30]. Distinct from HBV, HCV is an RNA virus and does not integrate into the host genome and replicates within the hepatocyte cytoplasm. HCV-related HCCs were found to enrich inactivating mutation of ARID2 gene [53], silencing of *CDKN2A* promoter, and *TERT* promoter mutation [24].

Other potential environmental factors were also found to leave a distinct "molecular fingerprint" in HCC, for example, alcohol-related tumors were significantly enriched with inactive mutation of *ARID1A* and enriched with alterations of CTNNB1, TERT, CDKN2A, SMARCA2, and HFG [27, 28], and aflatoxin exposure was significantly related to *TP53 R249S* mutation [28, 54].

It is not uncommon that patients may be exposed to more than one risk factors related to HCC, and different environmental exposures often show a synergized effect in promoting the progression of hepatocarcinogenesis. For example, alcohol use doubles the risk of HCV-related HCC [55], and smoking increases the risk of alcoholism-related HCC [56]. Interestingly, evidence of synergistic interaction between different environmental factors could be found in molecular studies; for instance, HCC tumors related to aflatoxin B1 exposure and HBV infection shared similar genetic characteristics, recurrent TP53-R249S mutation, and high AFB1 signature [24], which correlated with clinical observations [57].

Individual Genetic Predispositions

Individual genetic predisposition (also called genetic susceptibility) reflects the collective effect of germline mutation(s); it influences the individual risk or tendency to develop disease and contributes to the heterogeneous biology of HCC [50, 58]. According to a number of genetic alterations involved, genetic predisposition could be further subclassified as "monogenic" and "polygenic."

Monogenic Germline Variance

Several monogenic predispositions related to increasing risk of HCC have been reported, and alpha-1 antitrypsin (AAT) deficiency [59], hereditary tyrosinemia type 1 [60], hemochromatosis [61], and porphyrias [62] are the most well-known.

AAT deficiency is caused by mutation of SERPINA1 gene, resulting in altered protease/antiprotease balance, and associated with an increased risk of HCC, particularly in male patients [59, 63]. Hereditary tyrosinemia type 1 is caused by mutation of FAH gene, which leads to accumulation of tyrosine catabolic intermediates in the liver due to defective tyrosine metabolism, and, subsequently, results in liver inflammation and HCC development. Hemochromatosis is an iron metabolism disorder related to the mutation of HFE gene; patients harboring C282Y mutation of HFE gene have excessive gastrointestinal iron absorption and storage in the liver

and many other organs. The risk of HCC in hemochromatosis patient is approximately 20-fold higher compared to the general population [61, 64]. Hepatic porphyrias are a group of diseases associated with abnormal heme biosynthesis; reduced free heme pool may increase the reactive oxygen species stress and mutation burden in patients with porphyria and consequently increases the risk of HCC [65]. Besides porphyrias, all abovementioned syndromes are inherited in an autosomal recessive fashion.

It is worth noting that HCC that developed in patients with germline mutation which we discussed here may have a different clinical presentation than those without, and their liver tumors tend to occur earlier. For example, 40% of patients with tyrosinemia type 1 develop HCC in their childhood [60]. Patients with AAT deficiency can develop pulmonary emphysema [59]. Some porphyria patients have neuropsychiatric symptoms [50]. On the other hand, we should also keep in mind that for most of the monogenetic syndromes, harboring genetic changes is not sufficient to drive the formation of HCC, suggesting a role of other modulating factors (environmental or genetic) [50].

Polygenic Risk Factors

Several conditions or diseases inherited as polygenic traits are associated with a higher risk of HCC. For example, patients with type 2 diabetes mellitus have a 2.5-fold increase of HCC risk [66], and patients with nonalcoholic steatohepatitis (NASH)-related cirrhosis have an annual 2.4–12.8% HCC incidence rate [67]. Other conditions, such as hypothyroidism, autoimmune hepatitis, and positive family history are also regarded as polygenic risk factors for HCC [50]. Apart from monogenetic risk factor, the incremental risk of HCC in patients with polygenetic predisposition is mediated by the combination of many genetic variations. Therefore, it is not surprised to see that these polygenetic conditions connect to hepatocarcinogenesis via multiple mechanisms. For instance, the development of HCC in patients with NASH may be attributed to obesity, insulin resistance, lipotoxicity, dysregulation of intestinal microflora, and genetic polymorphism [67]. Similar to monogenetic risk factor, polygenetic factors may interact and synergize with environmental factors in promoting hepatocarcinogenesis. For example, the risk of virus-related HCC is higher in patients comorbid with diabetes [68] and hypothyroidism [69].

Somatic Molecular Alterations

Somatic alterations account for 90% of molecular alterations occurring in the tumor genome. Taking the different biological function of each molecule into account, a vast amount of molecular alterations are the major contributors to tumor heterogeneity.

Characterizing Molecular Heterogeneity in HCC: A Rapidly Evolving Journey

Multiple molecular alterations and critical oncogenic signaling pathways had been identified in HCC tumors (as summarized in section "Molecular Alterations of HCC"). Each molecular feature, i.e., high vs. low CNVs or TP53 mutant vs. wild type, explains partly the trajectory of a tumor and could be taken as a reference to define heterogeneity. For example, Katoh et al. used the pattern of CNV to identify biologically distinct clusters among 87 HCC patients and showed that patients in the cluster with more dominant CNV features had worse survival [17]. Notably, different from some of the malignant diseases with strong molecular-alteration-driven phenotype, such as adenocarcinoma of the lung and epithelial growth factor receptor mutation [70] or estrogen-receptor-positive breast cancer [71], there is no dominant oncogenic molecular feature being recognized in HCC, meaning that it is difficult to define a homogenous patient or tumor clusters by single or few molecular features.

With the advent of bioinformatics and computational power, researchers are able to process and analyze a vast amount of data at the same time. Several studies showed that combining multiple "omics" data, namely, genomics, transcriptome, epigenome, and metabolome, could provide a better molecular classification in HCC and greatly enhanced our understanding of this complex disease [15, 20, 24, 72–77]. However, different omics platforms use very distinct methods to analyze different biological aspects of a subject, resulting in a huge inherited heterogeneity among the data and difficulty in combining them for analysis. To solve this problem, many studies chose to use a platform to produce a stable signature for clustering and match data generated from the other platforms to this clustering. Transcriptome profiling is widely adapted to identify stable molecular subtypes linking to tumor biology and clinical outcome in HCC [72, 74]. Evidence applying tumor transcriptome to define HCC subtypes linked to metastasis status and patient survival was first obtained by Ye et al. [78]. Lee et al. subsequently applied an unsupervised approach to define HCC molecular subtypes [79]. Boyault et al. defined six clusters among 65 samples according to their transcriptomic data and showed that these six clusters (G1–G6) link to distinct genotype and phenotype [72]. G1 tumors were typified by low copy number of HBV, AXIN1 mutation, younger age, higher serum level of AFP, and frequent origin from Africa. G2 tumors were associated with high HBV burden and mutations of PI3KCA and TP53. Similar to G2, G3 tumors were also associated with TP53 mutation but could be differentiated by lacking HBV infection and overexpression of cell cycle regulatory genes, such as CDC6, MAD2L1, CCNA2, and CCNE2. G4 was considered as a heterogeneous group that was comprised of both tumor and non-tumor sample, and a subgroup with TCF1 mutation was identified in G4. For the rest of the two clusters, G5 and G6 were both associated with activation of Wnt/β-catenin, but tumors of the G6 cluster presented with a higher degree of β-catenin activation and more satellite nodules around the main tumors based on pathologic analysis. Chaisaingmongkol et al. analyzed tran-

scriptome data obtained from 62 Thai HCC patients by consensus clustering method and identified three different clusters (C1–C3) linking to unique genomic and metabolomic features and patients' clinical outcomes [74]. More interestingly, in this study, the authors showed that the C1 and C2 signatures in HCC were shared with a subgroup of patients with cholangiocarcinoma in an ancestry-dependent manner (will be discussed in more detail in the following section).

A transcriptome meta-analysis that involves 603 HCC tumors identified three molecular subtypes (S1–S3) commonly observed across geographic regions and patient races/ethnicities [73]. S1 tumors are characterized with activation of transforming growth factor (TGF)-beta and Wnt pathways and associated with a more disseminative phenotype. S2 tumors are characterized by positivity of stemness markers such as *AFP* and *EPCAM*. S1 and S2 tumors collectively represent more aggressive tumors accompanied with more frequent *TP53* mutations. S3 tumors are more differentiated and less aggressive compared to S1/S2 tumors (indolent subtype), in which *CTNNB1*-mutated tumors are accumulated. Of note, histological variants and clinical variables are associated with molecular subtypes [80]. Steatohepatitic variant and immune cell infiltrates are more frequently observed in S1 tumors. S2 tumors are associated with a macrotrabecular/compact pattern, clear cell variant, and high serum AFP. S3 tumors are associated with microtrabecular and pseudoglandular patterns. Similar correlations were confirmed in subsequent studies [81, 82].

On the other hand, several studies demonstrated the feasibility to interrogate different omics data to identify molecular subtypes [24, 75, 76]. Woo et al. combined DNA copy number and DNA methylation pattern for molecular classification and identified three subtypes in HCC (C1–C3); C1 tumors were typified by the highest frequency of CNV and methylation; recurrent BAP1 mutation; higher expression of CA9, KRT19, EPCAM, and PROM; and worse clinical survival [75]. Another good example of multi-omics study in HCC is the TCGA cohort, which comprehensively studied hundreds of HCC patients using six different platforms, namely, exome sequencing, DNA copy number, mRNA sequencing, microRNA sequencing, methylomics, and proteomics [83]. In the TCGA cohort, three subtypes (iC1–iC3) were identified based on the results of copy number and methylation change of DNA, expressions of mRNA and miRNA, and protein array. Interestingly, this molecular classification not only differentiates the molecular features of tumors but also showed a strong link with important clinical features of patients. For example, iC1 tumors, characterized by low frequency of CTNNB1 mutation, TERT promoter mutation, and DNA-methylation-mediated DKN2A silencing, were clinically linked to younger age, Asian ethnicity, female gender, and normal body weight, and iC2 tumors tended to have low-grade differentiation and less microvascular invasion. Collectively, molecular heterogeneity in HCC has been demonstrated in several studies and in various cohorts. Comprehensive molecular profiling enhances our understanding of the oncogenic events relevant to the development and progression of HCC.

Molecular Similarity of HCC and Intrahepatic Cholangiocarcinoma

Most of our current knowledge of defining specific cancer types are primarily based on pathological findings; we ask where the tumor is found, what it looks like, and if it presents with specific markers. HCC and intrahepatic cholangiocarcinoma (iCCA), the two major types of primary liver cancer, were considered as two distinct diseases in terms of tumor origin, morphology, and clinical behavior [83–85]. Surprisingly, with the availability of large-scale genomic studies, common molecular features were found in a subset of patients with HCC or iCCA.

In the TCGA cohort, there were four HCC patients who presented with positive IDH1/2 mutations, a genetic alteration being more frequently seen in iCCA rather than HCC [24]. Besides confirming the histopathological presentations of these four tumors as pure HCC, the authors further showed that the IDH1/2 mutation linked to a unique transcriptome and miRNA signature, an aggressive tumor behavior, and worse clinical course, suggesting a novel subclass in HCC being identified. On the other hand, the work done by Chaisaingmongkol et al. also identified similar molecular features in these two diseases [74]. They used global transcriptome expression to define molecular classes in HCC and iCC and identified three subtypes of HCC and four subtypes of ICC. Intriguingly, they compared subtypes of HCC and iCCA and found that the C1 and C2 of HCC were biologically similar with that of iCCA. The C1 subtype was enriched with PLK1 and ECT2 mutation and associated with worse clinical outcomes, where the C2 subtype presented with link to obesity, T cell infiltration, bile acid metabolism, and better outcomes. They further validated the presence of the C1/2 signature in three different HCC cohorts and two iCCA cohorts and found that these two signatures could be identified in Asian, not Caucasian, HCC/iCCA patients.

The commonality of HCC and iCCA identified by the abovementioned studies highlights the value of large-scale genomic analysis in fully addressing cancer heterogeneity and associated distinct tumor biology in different patients.

Intratumoral Heterogeneity in HCC

Evolution of Intratumoral Heterogeneity

Intratumoral heterogeneity, referring to the variations among different tumor lesions and/or tumor cells within a single patient, is largely driven by genomic instability [49]. Unstable genome in tumor cells leads to the occurrence of a wide range of mutations and, as such, fosters genetic diversity and the generation of genetically different cancer cell clones. As a tumor expands or develops metastasis, these clones would compete for survival, leading to clonal evolution of a given lesion or host. Therefore, the architecture of tumor lesion is determined by a framework composed of clonal evolution, competition, and best-fit selection. Additionally,

it is worthy of note that intratumoral heterogeneity is not only limited to the uneven distribution of tumor clones across various lesions but also includes the dynamic changes of a given lesion (also termed as temporal heterogeneity) [49, 86].

Spectrum of Intratumoral Heterogeneity

Heterogeneity of Different Tumor Lesions

As we mentioned at the beginning, intratumoral heterogeneity in HCC was first characterized by Dr. Kojiro's group who found that 47.7% of patients harbor two or more subpopulations within one tumor [8]. Later, Dr. Weber's group analyzed 120 tumor areas obtained from 23 patients and found that 87% of patients had evidence of intratumoral heterogeneity defined by morphological presentation, immunohistochemical staining of a collection of liver cell markers, and mutation status of *TP53* and *CTNNB1* [87]. Notably, the percentage of detectable genetic variations among different lesions increased in patients with a larger tumor or advanced disease stage, suggesting that intratumoral heterogeneity evolves during tumor progression. In addition, 26% of patients in this study with morphologically distinct tumors showed no differences on the protein level and the mutation status of *TP53* and *CTNNB1*, suggesting the limitation of given methodology in fully addressing heterogeneity.

Using whole-genome sequencing to analyze 43 tumor lesions from 10 HCC patients, Xue et al. showed that all the patients in this cohort presented with evidence of intratumoral heterogeneity [88]. Importantly, the extent of intratumoral heterogeneity varied among different patients. By interrogating the features of somatic mutation, hepatitis B integrations, and copy number variations, the authors supported the branched evolution of different HCC clones. Compared to primary tumors, mutation patterns of intrahepatic metastasis or tumor thrombi were more distinct than that of satellite nodules.

Collectively, the abovementioned studies showed the presence of intratumor heterogeneity linking to distinct biology in HCC and suggested that analyzing a single lesion may be underrepresented.

Heterogeneity at Single-Cell Level

As mentioned at the beginning of this section, a single cancer cell can give rise to a distinct subpopulation (also termed clone). With tumor progression, cells within one clone may develop new mutations, leading to formation of different subclone(s). Therefore, within one tumor lesion, a cell per se may be different from each other, making single-cell study a must to fully address the intratumoral heterogeneity of a tumor.

Hou et al. were the first to report heterogeneity at the single-cell level in HCC; they used a single-cell triple omics sequencing technique, which simultaneously

analyze genome, DNA methylome, and transcriptome of a single cell, to analyze 25 single cells derived from an HCC patient [89]. Importantly, even in such a few number of cells being studied, they identified two subgroups of cells with distinct molecular features. The other work presented by Zheng et al. focused on exploring the heterogeneity of a specific cell population – cancer stem cell (CSC) – by analyzing 2595 CSCs obtained from one HCC patient or enriched from HuH1 and HuH7 cells [90]. Using single-cell RNA sequencing to characterize transcriptome features or flow cytometry to determine the intensities of stem cell markers expressed on cell surfaces, they showed a huge heterogeneity among these cells. Importantly, the transcriptome signatures obtained from different subpopulations were associated with the outcome of patients in different HCC cohorts. The other thing worthy of note was that if the authors mixed more than 100 tumor cells and conducted genomic analysis of this cell mixture (simulation of bulk sample), the variations identified at the single-cell level could not be recaptured, suggesting the data we obtained from bulky tumor samples might be biased in a certain degree.

Stem Cell Feature and Heterogeneity in HCC

CSC, also termed as tumor-initiating cell, refers to a subset of cancer cells with self-renewal and differentiation capabilities. Notably, the presence of CSCs in tumor is not only a demonstration of intratumor heterogeneity (a subset of cells that are functionally distinct from the rest), but it also promotes repopulation of tumor cells, resulting in greater intratumoral heterogeneity [91].

In HCC, the presence of CSC can be phenotypically identified by specific markers, such as CD133, EpCAM, and CD44, or functionally defined by the capability of tumor initiation and asymmetric differentiation [92]. Ma et al. isolated CD133-high and CD133-low expressed cells from two human HCC cell lines and showed that CD133+ HCC cells confer higher proliferation and chemoresistance [93]. In concordance, Zheng et al. showed that a specific subgroup of cells positive for CD133 and CD44 was identified in a 2-AAF-induced rat liver cancer model, and this specific $CD133^{+}CD44^{+}$ cell clone could expand and differentiate into bi-lineage cell types [94]. The ability of bi-lineage differentiation indicates that CSC can initiate branching evolution, denoting the emergence and divergent propagation of multiple subclonal tumor cell populations from a common ancestor [49]. Compared with linear evolution (sequential genetic alterations and survival-of-the-fitness selection convey a linear model of clonal evolution), branching evolution is more likely to create a more heterogeneous tumor, which had been evidenced in HCC and many other types of cancers [49, 88, 95].

On the other hand, a growing body of literature suggest that the stem cell signature is an important feature that defines intertumoral heterogeneity in HCC. Yamashita et al. showed that HCC patients could be subclassified into four groups according to the expression levels of two hepatic stem cell-associated marker, epithelial cell adhesion molecule (EpCAM) and α-fetoprotein (AFP); patients with hepatic stem

cell-like HCCs ($EpCAM^+$, AFP^+) and hepatocytic progenitor-like HCCs ($EpCAM^-$, AFP^+) had worse survival in comparison to others [96]. More importantly, tumors with the hepatic stem cell-like signature were characterized by unique molecular features shown at global transcriptome, miRNA, and metabolomic levels [48, 97].

Collectively, CSCs affect clonal architecture in a tumor, permitting the development and progression of tumor heterogeneity in HCC. Tracing the life path of CSCs provides an important scope to identify critical mechanisms driving tumor progression in HCC.

Immune Heterogeneity in HCC

"No man is an island," as stated in the famous work by John Donne; cancer cells are within a complex community comprised by various immune and stromal cells. Tumor cells interact with and are being affected by their surrounding cells, suggesting that the tumor-associated microenvironment could be an important driver of tumor heterogeneity [98]. A major portion of the tumor-associated microenvironment is immune cells, which interact frequently with tumor cells. The cross talk between tumor and immune cells is complicated; the immune system had been implicated in preventing and promoting tumor growth, and on the other hand, tumor cells were shown to be able to shape the immune contexture and escape immune attack through tumor progression [98]. Notably, the effects of the immune system on a tumor are heterogeneous both among patients and lesions.

In the TCGA cohort, about 22% of HCC patients displayed high or moderate levels of lymphocyte infiltration, and about one-third of patients exhibited high expression of immune markers, such as CTLA-4 and other immune checkpoints [24]. Additionally, the variations of immune contexture were not only observed between different patients, but evidence of a distinct tumor immune microenvironment coexisting within a single individual had been shown in ovarian cancer [99].

Taken together, tumor-associated immune microenvironment is highly heterogeneous and tightly connects with the development and progression of a tumor. Addressing the discrepancies of the immune system is needed to fully acknowledge the complex tumor ecosystem in HCC.

Adapting Tumor Heterogeneity to Personalized Treatment in HCC

Hepatocarcinogenesis is a highly complex multistep process driven by host genome alterations and chronic liver inflammation. Therefore, the most common feature of HCC patients may be "heterogeneous." Recognizing genetic features/classes to identify a critical target for drug development, to help in outcome prediction, and to

guide personalized medicine had shown to be a successful strategy in breast cancer, lung cancer, and many other malignant diseases [70, 71, 100]. With the accumulation of large-scale genomic studies in the recent decade, our understanding of HCC genome evolution, potential druggable molecular alterations, and the dynamic interaction between HCC cells and microenvironment has increased significantly. However, the improvement of knowledge has not yet been able to change the paradigm of HCC treatment like what had been shown in other malignant diseases.

Several reasons may be responsible for this disappointing situation. First, molecular heterogeneity among HCC patients was insufficiently acknowledged in the commonly adapted clinical trial design. Till present, most of the published clinical trials in HCC are still based on the "all-comer" design to test their compound of interest, including molecular target agents. The unmeasured patient heterogeneity could be a significant confounder to the results of clinical trials. A good example is the development journey of ramucirumab, a monoclonal antibody against vascular endothelial growth factor receptor 2. In its first phase III trial (REACH trial), ramucirumab was tested as a second-line treatment for HCC in a nonselected patient cohort, and the results failed to demonstrate any survival benefit in patients treated with ramucirumab in comparison to placebo [101]. But the researchers found that the response rates of patients with high AFP levels were significantly higher in the post hoc analysis of REACH trial, leading to a follow-up trial that focused only on patients with high serum AFP level. Encouragingly, researchers successfully showed a 55% of recurrence risk reduction in this subgroup of patients, which was originally diluted in a mixed patient papulation [102]. Currently, there are some ongoing studies being designed in a biomarker enrichment base, such as LY2157299 in patients with high AFP (NCI01246989) and JNJ-42756293 in patients with fibroblast growth factor 19 amplification. Additionally, there are two innovated models of clinical trial designs, the basket study design and the umbrella study design, being proposed to help researchers address intertumoral heterogeneity among patients and to improve the efficiency in testing potential druggable molecular alterations in cancer patients. The "basket trial" is designed to test the effect of a single intervention on a specific molecular mutation/features regardless of cancer types, while the "umbrella trial" is designed to test the potencies of different drugs on different mutations under the "same disease umbrella" [103, 104]. Several large ongoing clinical trials, such as the NCI-MATCH study, adapted these novel study structures to design their protocols, and some of them had already released good results [105, 106].

Second, intratumoral heterogeneity, particularly the treatment-induced dynamic clonal changes, was shown to be a critical reason for primary resistance and relapse to target therapy in many types of cancers [49, 107], but this issue has not been addressed in current HCC clinical trials. Part of the reasons could be the difficulty of obtaining adequate tissues for molecular profiling, particularly obtaining samples of multiple lesions from a patient or sequential sampling. Alternatively, the use of less invasive tests, such as circulating tumor cells, circulating DNA, or multiparametric magnetic resonance imaging, may be more feasible to be adapted into clinical trial or even routine clinical practice and help researchers to capture the

diversity across different tumor lesions and/or the dynamic changes along with molecular interventions and disease progression [108, 109].

Lastly, similar to drug sensitivity, drug tolerability is heterogeneous among patients. With a high incidence of liver cirrhosis, the individual variations of drug metabolism could magnify the differences of individual drug tolerability. So far, how to predict the occurrence and/or the severity of treatment-associated adverse effects has not yet been fully addressed in HCC. In patients with renal cell carcinoma receiving sorafenib treatment, Qin et al. showed that a polymorphism of VEGF, VEGF rs2010963 CG + GG genotype, was significantly linked to a higher risk of hand-foot syndrome, a common side effect associated with sorafenib [110]. Whether this or other genomic variations may be associated with the safety profile of sorafenib in HCC remained unclear, and more studies are warranted in the future.

Conclusion

HCC is a disease of great genetic diversity. With numerous tools available today, hundreds to thousands of molecular alterations could be detected within a liver tumor, but how to capture the story leading to tumor development and progression remains to be a big challenge. Recognizing molecular heterogeneity does not aim to find differences that separate patients but, by contrast, to identify critical features to unite patients into a relatively homogeneous subgroup. The diagnosis of HCC in this "omics era" should not be restricted to image-based criteria. Interrogating molecular profiling in different patients and, even, at various regions of a given tumor is the foundation of precision medicine. Full recognition of tumor heterogeneity is required not only to improve diagnosis and outcome prediction but also, more importantly, to allow researcher and clinician to design more effective anticancer therapies to every HCC patient.

Acknowledgement This work is supported by the Intramural Research Program of the Center for Cancer Research of the U.S. National Cancer Institute (Z01 BC 01313 and Z01 BC 010877) and Taipei Veterans General Hospital-National Yang-Ming University Excellent Physician Scientist Cultivation Program.

References

1. Wong MCS, Jiang JY, Goggins WB, Liang M, Fang Y, Fung FDH, et al. International incidence and mortality trends of liver cancer: a global profile. Sci Rep. 2017;7:45846. https://doi.org/10.1038/srep45846. https://www.nature.com/articles/srep45846#supplementary-information.
2. ClinicalTrials.gov. Interventional studies | hepatocellular carcinoma. Bethesda (MD): National Library of Medicine (US); 2018. https://www.clinicaltrials.gov/ct2/results?cond=Hepatocellular+Carcinoma&age_v=&gndr=&type=Intr&rslt=&Search=Apply. Accessed June 14 2018.

3. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90.
4. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;389(10064):56–66. https://doi.org/10.1016/S0140-6736(16)32453-9.
5. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. 2018;391(10126):1163–73. https://doi.org/10.1016/S0140-6736(18)30207-1.
6. Merriam-Webster. "Heterogeneity.". 2018. www.merriam-webster.com/dictionary/heterogeneity. Accessed 14 June 2018.
7. Edmondson H. Tumors of the liver and intrahepatic bile ducts, atlas of tumors pathology. Washington, DC: Armed Forces Institute of Pathology; 1958.
8. Kenmochi K, Sugihara S, Kojiro M. Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small HCC. Liver. 1987;7(1):18–26.
9. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007;446(7132):153–8.
10. Nagy R, Sweet K, Eng C. Highly penetrant hereditary cancer syndromes. Oncogene. 2004;23:6445. https://doi.org/10.1038/sj.onc.1207714.
11. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science (New York, NY). 2015;349(6255):1483–9. https://doi.org/10.1126/science.aab4082.
12. Shibata T, Aburatani H. Exploration of liver cancer genomes. Nat Rev Gastroenterol Hepatol. 2014;11(6):340.
13. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet. 2006;7(2):85–97. https://doi.org/10.1038/nrg1767.
14. Shlien A, Malkin D. Copy number variations and cancer. Genome Med. 2009;1(6):62. https://doi.org/10.1186/gm62.
15. Wang K, Lim HY, Shi S, Lee J, Deng S, Xie T, et al. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. Hepatology. 2013;58(2):706–17. https://doi.org/10.1002/hep.26402.
16. Schlaeger C, Longerich T, Schiller C, Bewerunge P, Mehrabi A, Toedt G, et al. Etiology-dependent molecular mechanisms in human hepatocarcinogenesis. Hepatology. 2008;47(2):511–20.
17. Katoh H, Ojima H, Kokubu A, Saito S, Kondo T, Kosuge T, et al. Genetically distinct and clinically relevant classification of hepatocellular carcinoma: putative therapeutic targets. Gastroenterology. 2007;133(5):1475–86. https://doi.org/10.1053/j.gastro.2007.08.038.
18. Zhao X, Parpart S, Takai A, Roessler S, Budhu A, Yu Z, et al. Integrative genomics identifies YY1AP1 as an oncogenic driver in EpCAM(+) AFP(+) hepatocellular carcinoma. Oncogene. 2015;34(39):5095–104. https://doi.org/10.1038/onc.2014.438.
19. Cronin KA, Lake AJ, Scott S, Sherman RL, Noone AM, Howlader N, et al. Annual report to the nation on the status of cancer, Part I: national cancer statistics. Cancer. 2018; https://doi.org/10.1002/cncr.31551.
20. Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet. 2014;46(12):1267–73. https://doi.org/10.1038/ng.3126.
21. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, Ishikawa S, et al. High-resolution characterization of a hepatocellular carcinoma genome. Nat Genet. 2011;43(5):464–9.
22. Fujimoto A, Furuta M, Totoki Y, Tsunoda T, Kato M, Shiraishi Y, et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nat Genet. 2016;48(5):500–9. https://doi.org/10.1038/ng.3547.

23. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet. 2012;44:760–4.
24. The Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;169(7):1327–41 e23. https://doi.org/10.1016/j.cell.2017.05.046.
25. Poon SL, Pang ST, McPherson JR, Yu W, Huang KK, Guan P, et al. Genome-wide mutational signatures of aristolochic acid and its application as a screening tool. Sci Transl Med. 2013;5(197):197ra01. https://doi.org/10.1126/scitranslmed.3006086.
26. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature. 1991;350:427–8.
27. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet. 2012;44(6):694–8. https://doi.org/10.1038/ng.2256.
28. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet. 2015;47(5):505–11. https://doi.org/10.1038/ng.3252. http://www.nature.com/ng/journal/v47/n5/abs/ng.3252.html#supplementary-information.
29. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27. https://doi.org/10.1056/NEJMra1001683.
30. Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet. 2012;44(7):765.
31. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003;33(Suppl):245–54.
32. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007;133(2):647–58. https://doi.org/10.1053/j.gastro.2007.05.022.
33. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene. 2009;28(40):3526–36. https://doi.org/10.1038/onc.2009.211.
34. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1):a001008. https://doi.org/10.1101/cshperspect.a001008.
35. Soussi T. The TP53 gene network in a postgenomic era. Hum Mutat. 2014;35(6):641–2. https://doi.org/10.1002/humu.22562.
36. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature. 1998;396(6706):84–8. https://doi.org/10.1038/23962.
37. Ellis L, Atadja PW, Johnstone RW. Epigenetics in cancer: targeting chromatin modifications. Mol Cancer Ther. 2009;8(6):1409–20. https://doi.org/10.1158/1535-7163.mct-08-0860.
38. Baylin SB, Jones PA. A decade of exploring the cancer epigenome – biological and translational implications. Nat Rev Cancer. 2011;11:726–34.
39. Carracedo A, Pandolfi PP. The PTEN–PI3K pathway: of feedbacks and cross-talks. Oncogene. 2008;27:5527. https://doi.org/10.1038/onc.2008.247.
40. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci. 1996;93(25):14960–5. https://doi.org/10.1073/pnas.93.25.14960.
41. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008;7(1):11–20. https://doi.org/10.1016/j.cmet.2007.10.002.

42. Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? Nat Rev Cancer. 2016;16:635. https://doi.org/10.1038/nrc.2016.77.
43. Li L, Che L, Tharp KM, Park HM, Pilo MG, Cao D, et al. Differential requirement for de novo lipogenesis in cholangiocarcinoma and hepatocellular carcinoma of mice and humans. Hepatology. 2016;63(6):1900–13. https://doi.org/10.1002/hep.28508.
44. Kim MJ, Choi YK, Park SY, Jang SY, Lee JY, Ham HJ, et al. PPARdelta Reprograms Glutamine Metabolism in Sorafenib-Resistant HCC. Mol Cancer Res. 2017;15(9):1230–42. https://doi.org/10.1158/1541-7786.mcr-17-0061.
45. Beyoglu D, Idle JR. Metabolomics and its potential in drug development. Biochem Pharmacol. 2013;85(1):12–20. https://doi.org/10.1016/j.bcp.2012.08.013.
46. Patterson AD, Maurhofer O, Beyoglu D, Lanz C, Krausz KW, Pabst T, et al. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. Cancer Res. 2011;71:6590–600.
47. Yin P, Wan D, Zhao C, Chen J, Zhao X, Wang W, et al. A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. Mol Biosyst. 2009;5:868–76.
48. Budhu A, Roessler S, Zhao X, Yu Z, Forgues M, Ji J, et al. Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. Gastroenterology. 2013;144(5):1066–75 e1. https://doi.org/10.1053/j.gastro.2013.01.054.
49. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol. 2018;15(2):81–94. https://doi.org/10.1038/nrclinonc.2017.166.
50. Dragani TA. Risk of HCC: genetic heterogeneity and complex genetics. J Hepatol. 2010;52(2):252–7. https://doi.org/10.1016/j.jhep.2009.11.015.
51. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer. 2013;13(2):123–35. https://doi.org/10.1038/nrc3449.
52. Neuveut C, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. J Hepatol. 2010;52(4):594–604. https://doi.org/10.1016/j.jhep.2009.10.033.
53. Li M, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, et al. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. Nat Genet. 2011;43(9):828–9. https://doi.org/10.1038/ng.903.
54. Villar S, Ortiz-Cuaran S, Abedi-Ardekani B, Gouas D, Nogueira da Costa A, Plymoth A, et al. Aflatoxin-induced TP53 R249S mutation in hepatocellular carcinoma in Thailand: association with tumors developing in the absence of liver cirrhosis. PLoS One. 2012;7(6):e37707. https://doi.org/10.1371/journal.pone.0037707.
55. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology. 2004;127(5 Suppl 1):S87–96.
56. Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, Adami HO, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer. 2000;85(4):498–502.
57. Humans IWGotEoCRt. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr Eval Carcinog Risks Hum. 2002;82:1–556.
58. Frank SA. Genetic predisposition to cancer — insights from population genetics. Nat Rev Genet. 2004;5:764. https://doi.org/10.1038/nrg1450.
59. Fairbanks KD, Tavill AS. Liver disease in alpha 1-antitrypsin deficiency: a review. Am J Gastroenterol. 2008;103(8):2136–41; quiz 42. https://doi.org/10.1111/j.1572-0241.2008.01955.x.
60. Weinberg AG, Mize CE, Worthen HG. The occurrence of hepatoma in the chronic form of hereditary tyrosinemia. J Pediatr. 1976;88(3):434–8.
61. Elmberg M, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. Gastroenterology. 2003;125(6):1733–41.

62. Fracanzani AL, Taioli E, Sampietro M, Fatta E, Bertelli C, Fiorelli G, et al. Liver cancer risk is increased in patients with porphyria cutanea tarda in comparison to matched control patients with chronic liver disease. J Hepatol. 2001;35(4):498–503.
63. Elzouki AN, Eriksson S. Risk of hepatobiliary disease in adults with severe alpha 1-antitrypsin deficiency (PiZZ): is chronic viral hepatitis B or C an additional risk factor for cirrhosis and hepatocellular carcinoma? Eur J Gastroenterol Hepatol. 1996;8(10):989–94.
64. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology. 2004;127(5 Suppl 1):S79–86.
65. Andersson C, Bjersing L, Lithner F. The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. J Intern Med. 1996;240(4):195–201. https://doi.org/10.1046/j.1365-2796.1996.21847000.x.
66. Mantovani A, Targher G. Type 2 diabetes mellitus and risk of hepatocellular carcinoma: spotlight on nonalcoholic fatty liver disease. Ann Transl Med. 2017;5(13):270. https://doi.org/10.21037/atm.2017.04.41.
67. Cholankeril G, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma in non-alcoholic steatohepatitis: current knowledge and implications for management. World J Hepatol. 2017;9(11):533–43. https://doi.org/10.4254/wjh.v9.i11.533.
68. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557–76. https://doi.org/10.1053/j.gastro.2007.04.061.
69. Hassan MM, Kaseb A, Li D, Patt YZ, Vauthey JN, Thomas MB, Curley SA, Spitz MR, Sherman SI, Abdalla EK, Davila M. Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. Hepatology. 2009;49(5):1563–70. https://doi.org/10.1002/hep.22793.
70. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005;352(8):786–92.
71. van't Veer LJ, Dai H, Van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415(6871):530–6.
72. Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology. 2007;45(1):42–52. https://doi.org/10.1002/hep.21467.
73. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res. 2009;69(18):7385–92. https://doi.org/10.1158/0008-5472.CAN-09-1089.
74. Chaisaingmongkol J, Budhu A, Dang H, Rabibhadana S, Pupacdi B, Kwon SM, et al. Common molecular subtypes among Asian hepatocellular carcinoma and cholangiocarcinoma. Cancer Cell. 2017;32(1):57–70 e3. https://doi.org/10.1016/j.ccell.2017.05.009.
75. Woo HG, Choi J-H, Yoon S, Jee BA, Cho EJ, Lee J-H, et al. Integrative analysis of genomic and epigenomic regulation of the transcriptome in liver cancer. Nat Commun. 2017;8(1):839. https://doi.org/10.1038/s41467-017-00991-w.
76. Chaudhary K, Poirion OB, Lu L, Garmire LX. Deep learning-based multi-omics integration robustly predicts survival in liver cancer. Clin Cancer Res. 2018;24(6):1248–59. https://doi.org/10.1158/1078-0432.ccr-17-0853.
77. Xue R, Li J, Bai F, Wang X, Ji J, Lu Y. A race to uncover a panoramic view of primary liver cancer. Cancer Biol Med. 2017;14(4):335–40. https://doi.org/10.20892/j.issn.2095-3941.2017.0112.
78. Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, et al. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat Med. 2003;9(4):416–23.
79. Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology. 2004;40(3):667–76. https://doi.org/10.1002/hep.20375.

80. Tan PS, Nakagawa S, Goossens N, Venkatesh A, Huang T, Ward SC, et al. Clinicopathological indices to predict hepatocellular carcinoma molecular classification. Liver Int. 2016;36(1):108–18. https://doi.org/10.1111/liv.12889.
81. Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouze E, Blanc JF, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. J Hepatol. 2017;67(4):727–38. https://doi.org/10.1016/j.jhep.2017.05.014.
82. Kurebayashi Y, Ojima H, Tsujikawa H, Kubota N, Maehara J, Abe Y, et al. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. Hepatology. 2018;68(3):1025–41. https://doi.org/10.1002/hep.29904.
83. Lau SK, Prakash S, Geller SA, Alsabeh R. Comparative immunohistochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma. Hum Pathol. 2002;33(12):1175–81. https://doi.org/10.1053/hupa.2002.130104.
84. Chen LD, Xu HX, Xie XY, Xie XH, Xu ZF, Liu GJ, et al. Intrahepatic cholangiocarcinoma and hepatocellular carcinoma: differential diagnosis with contrast-enhanced ultrasound. Eur Radiol. 2010;20(3):743–53. https://doi.org/10.1007/s00330-009-1599-8.
85. Man XB, Tang L, Zhang BH, Li SJ, Qiu XH, Wu MC, et al. Upregulation of Glypican-3 expression in hepatocellular carcinoma but downregulation in cholangiocarcinoma indicates its differential diagnosis value in primary liver cancers. Liver Int. 2005;25(5):962–6. https://doi.org/10.1111/j.1478-3231.2005.01100.x.
86. Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer. 2013;108:479. https://doi.org/10.1038/bjc.2012.581.
87. Friemel J, Rechsteiner M, Frick L, Böhm F, Struckmann K, Egger M, et al. Intratumor heterogeneity in hepatocellular carcinoma. Clin Cancer Res. 2015;21(8):1951–61.
88. Xue R, Li R, Guo H, Guo L, Su Z, Ni X, et al. Variable intra-tumor genomic heterogeneity of multiple lesions in patients with hepatocellular carcinoma. Gastroenterology. 2016;150(4):998–1008. https://doi.org/10.1053/j.gastro.2015.12.033.
89. Hou Y, Guo H, Cao C, Li X, Hu B, Zhu P, et al. Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. Cell Res. 2016;26(3):304–19. https://doi.org/10.1038/cr.2016.23.
90. Zheng H, Pomyen Y, Hernandez MO, Li C, Livak F, Tang W, et al. Single cell analysis reveals cancer stem cell heterogeneity in hepatocellular carcinoma. Hepatology. 2018; https://doi.org/10.1002/hep.29778.
91. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105–11. https://doi.org/10.1038/35102167.
92. Nio K, Yamashita T, Kaneko S. The evolving concept of liver cancer stem cells. Mol Cancer. 2017;16:4. https://doi.org/10.1186/s12943-016-0572-9.
93. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene. 2008;27:1749–58.
94. Zheng YW, Tsuchida T, Shimao T, Li B, Takebe T, Zhang RR, et al. The CD133+CD44+ precancerous subpopulation of oval cells is a therapeutic target for hepatocellular carcinoma. Stem Cells Dev. 2014;23(18):2237–49. https://doi.org/10.1089/scd.2013.0577.
95. Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012;481(7381):306–13. https://doi.org/10.1038/nature10762.
96. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res. 2008;68(5):1451–61.
97. Ji J, Wang XW. Identification of cancer stem cell-related microRNAs in hepatocellular carcinoma. Methods Mol Biol. 2012;826:163–75.
98. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases — elimination, equilibrium and escape. Curr Opin Immunol. 2014;27:16–25. https://doi.org/10.1016/j.coi.2014.01.004.

99. Jimenez-Sanchez A, Memon D, Pourpe S, Veeraraghavan H, Li Y, Vargas HA, et al. Heterogeneous tumor-immune microenvironments among differentially growing metastases in an ovarian cancer patient. Cell. 2017;170(5):927–38.e20. https://doi.org/10.1016/j.cell.2017.07.025.
100. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351(27):2817–26.
101. Zhu AX, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol. 2015;16(7):859–70. https://doi.org/10.1016/S1470-2045(15)00050-9.
102. Zhu AX, Galle PR, Kudo M, Finn RS, Qin S, Xu Y, et al. A study of ramucirumab (LY3009806) versus placebo in patients with hepatocellular carcinoma and elevated baseline alpha-fetoprotein (REACH-2). J Clin Oncol. 2018;36(4_suppl):TPS538-TPS. https://doi.org/10.1200/JCO.2018.36.4_suppl.TPS538.
103. Cunanan KM, Iasonos A, Shen R, Begg CB, Gönen M. An efficient basket trial design. Stat Med. 2017;36(10):1568–79. https://doi.org/10.1002/sim.7227.
104. Renfro LA, Sargent DJ. Statistical controversies in clinical research: basket trials, umbrella trials, and other master protocols: a review and examples. Ann Oncol. 2017;28(1):34–43. https://doi.org/10.1093/annonc/mdw413.
105. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20. https://doi.org/10.1056/NEJMoa1500596.
106. Do K, O'Sullivan Coyne G, Chen AP. An overview of the NCI precision medicine trials-NCI MATCH and MPACT. Chin Clin Oncol. 2015;4(3):31. https://doi.org/10.3978/j.issn.2304-3865.2015.08.01.
107. Somasundaram R, Villanueva J, Herlyn M. Intratumoral heterogeneity as a therapy resistance mechanism: role of melanoma subpopulations. Adv Pharmacol (San Diego, Calif). 2012;65:335–59. https://doi.org/10.1016/B978-0-12-397927-8.00011-7.
108. Hectors SJ, Wagner M, Bane O, Besa C, Lewis S, Remark R, et al. Quantification of hepatocellular carcinoma heterogeneity with multiparametric magnetic resonance imaging. Sci Rep. 2017;7(1):2452. https://doi.org/10.1038/s41598-017-02706-z.
109. Sun Y-F, Guo W, Xu Y, Shi Y-H, Gong Z-J, Ji Y, et al. Circulating tumor cells from different vascular sites exhibit spatial heterogeneity in epithelial and mesenchymal composition and distinct clinical significance in hepatocellular carcinoma. Clin Cancer Res. 2018;24(3):547–59. https://doi.org/10.1158/1078-0432.ccr-17-1063.
110. Qin C, Cao Q, Li P, Wang S, Wang J, Wang M, et al. The influence of genetic variants of sorafenib on clinical outcomes and toxic effects in patients with advanced renal cell carcinoma. Sci Rep. 2016;6:20089. https://doi.org/10.1038/srep20089.

Chapter 15
Stromal and Immune Drivers of Hepatocarcinogenesis

Antonio Saviano, Natascha Roehlen, Alessia Virzì, Armando Andres Roca Suarez, Yujin Hoshida, Joachim Lupberger, and Thomas F. Baumert

Introduction

The liver is a multifunctional organ that plays a key role in metabolism and detoxification as well as in regulation of immune response and tolerance. The liver is physiologically exposed to many pathogens and toxic substances derived from the gut and has the largest population of resident macrophages (i.e., Kupffer cells, KCs) in the body and a high prevalence of natural killer cells (NK), natural killer T cells (NKT), and T cells. In normal conditions, the liver removes a large amount of microbes and pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) and maintains an immunosuppressive environment [1].

Following chronic hepatocyte damage, immune and stromal cells modify a liver environment, which triggers chronic inflammation and ultimately promotes hepatocellular carcinoma (HCC) [2]. Indeed, independently from the etiology, chronic liver disease is characterized by a deregulation in the liver immune network

The original version of this chapter was revised. The correction to this chapter can be found at https://doi.org/10.1007/978-3-030-21540-8_17

* Antonio Saviano and Natascha Roehlen are co-first authors of this chapter

A. Saviano · T. F. Baumert (✉)
Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Pôle Hépato-digestif, Institut Hopitalo-Universitaire, Hôpitaux Universitaires, Strasbourg, France
e-mail: thomas.baumert@unistra.fr

N. Roehlen · A. Virzì · A. A. Roca Suarez · J. Lupberger
Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Université de Strasbourg, Strasbourg, France

Y. Hoshida
Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, Division of Digestive and Liver Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_15

that stimulates cellular stress and death favoring liver fibrosis, hepatocyte proliferation, and epithelial-to-mesenchymal transition (EMT) [2]. A combination of EMT, genetic mutations, and epigenetic alterations that accumulate during cell proliferation is the most important driver of hepatocarcinogenesis [3].

Once HCC has developed, liver microenvironment greatly affects tumor progression and response to therapy [4]. This is the reason why gene expression signatures in liver tissues adjacent to the HCC—and the not in tumor itself—highly correlate with long-term survival of patients with liver fibrosis [5]. Similarly, HCC infiltration by non-parenchymal cells (e.g., regulatory T cells, T_{reg}) has been associated with tumor progression [5–8]. New therapies targeting liver microenvironment are recently developed or under clinical investigation for both chronic liver disease (e.g., nonalcoholic steatohepatitis, NASH) and HCC.

Hence, liver microenvironment plays an essential role in both hepatocarcinogenesis and tumor progression and it is an important therapeutic target for HCC prevention and treatment.

From Chronic Inflammation to Hepatocellular Carcinoma

HCC almost universally evolves on the background of chronic liver inflammation and liver fibrosis [9]. Chronic hepatocyte cell injury induces activation of the immune system that initiates and supports chronic inflammation by generation of proinflammatory cytokines and chemokines and activation of hepatic stellate cells (HSCs), finally resulting in liver fibrosis, cirrhosis, and cancer [10] (Fig. 15.1).

During chronic infections (e.g., hepatitis B virus, HBV, or hepatitis C virus, HCV) as well as metabolic (e.g., NASH) or toxic diseases (e.g., alcoholic steatohepatitis, ASH), immune cells—first of all KCs—are activated by the release of PAMPs and DAMPs produced by hepatocyte apoptosis and death. Activated KCs present viral antigens to T cells and/or secrete cytokines and chemokines that recruit circulating monocytes, lymphocytes, and neutrophils [11]. Proinflammatory signals are mainly mediated by the accumulation of tumor necrosis factor alpha (TNF-α); interleukins (IL) such as IL-6, IL-1β, IL-2, IL-7, IL-15, IL-17; C-C motif chemokine ligand 2 (CCL2); and interferon gamma (IFN-ɣ).

Following activation by antigen-presenting cells, T cells and especially T-helper 17 (Th17) cells and the mucosal-associated invariant T (MAIT) cells are major promoters of liver inflammation primarily by secretion of IL-17 [12, 13]. IL-17 secreted by T cells as well as transforming growth factor beta 1 (TGF-β1) and platelet-derived growth factor subunit B (PDGF-B) secreted by KCs and monocyte-derived macrophages are able to activate and differentiate HSC into collagen-producing myofibroblasts [12, 13]. Finally, also DAMPs can directly activate HSC and participate in fibrosis [7, 14]. HSC-derived myofibroblasts account for abnormal production of collagen in the liver and are main components of the hepatic precancerous microenvironment [15].

The inflammatory microenvironment causes hepatocellular stress, accompanied by epigenetic modifications, mitochondrial alterations, DNA damage, and

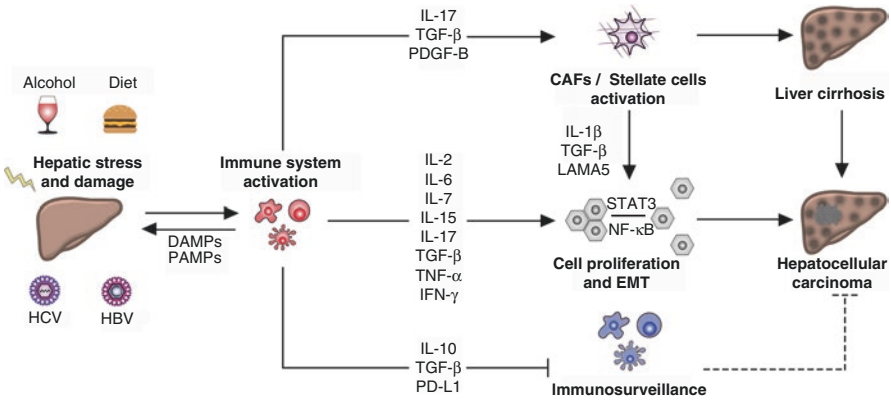

Fig. 15.1 Chronic inflammation is a pan-etiological driver of hepatocarcinogenesis. Hepatocarcinogenesis can be induced by multiple etiological and environmental conditions. Chronic HBV and HCV infections, as well as chronic alcohol abuse and metabolic syndrome trigger the activation of the innate immune system via release of Damage-Associated Molecular Patterns (DAMPs) and Pathogen Associated Molecular Patterns (PAMPs). The persistent dysregulation of the immunological network of the liver, promoted by the secretion of pro-inflammatory cytokines/chemokines (e.g. IL-2, IL-6, IL-7, IL-15, IL-17, TGF-β, TNF-α, IFN-γ), leads to cells death, compensatory hepatocellular proliferation, activation of cancer-associated fibroblasts (CAFs) and hepatic stellate cells (HSCs) as well as epithelial-tomesenchymal transition (EMT). Moreover, sustained necro-inflammatory status attenuates immune-surveillance and anti-tumor immune response, by secretion of anti-inflammatory molecules (e.g. IL-10, TGF-β, PD-L1). In addition, the activation of HSCs contributes significantly to cell proliferation (by the release of IL-1β, TGF-β and LAMA5) and cirrhosis. In conclusion, cellular proliferation and EMT, further sustained by STAT3/NF-κB pathway activation, cirrhosis and impaired immunosurveillance activity collectively contribute to HCC development

chromosomal alterations that determine cell transformations [7]. Inflammation has been shown to upregulate nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) thereby affecting cell proliferation, survival, angiogenesis, and chemotaxis [16–18]. STAT3 is further induced by several other cytokines and growth factors that are known to be upregulated under conditions of chronic liver inflammation [19]. Regarding chronic HBV and HCV infection, upregulation of the cytokines lymphotoxin beta and TNF-α in $CD4^+$ and $CD8^+$ T cells has been shown to promote hepatocarcinogenesis [20, 21].

Collectively, persistence of infection by hepatotropic viruses or toxic condition may cause a chronic inflammatory state, accompanied by continual cell death and promotion of compensatory tissue repair mechanisms, finally resulting in liver cirrhosis and cell transformation. Since chronic inflammation induces impaired immune surveillance due to exhausted T cells, chronic inflammatory liver status not only provokes cell transformation but also attenuates physiological antitumor defense mechanisms by the immune system. Thus, tumor cell attack by cytolytic T cells is weakened in chronic inflammatory liver tissue and HCC microenvironment [22–24].

Moreover, upregulation of immunosuppressive T_{reg} cells has been related to chronic inflammation associated with attenuated immune surveillance contributing to risk of HCC development [25, 26]. The inducible type 1 T regulatory (Tr1) cells

possess many immunosuppressive functions by secretion of the cytokines IL-10 and TGF-β, as well as by expression of the checkpoint inhibitors cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD1) on the cell surface [27–29]. T_{reg} or KC-secreted IL-10 was reported to reduce immune surveillance by suppressing macrophage activation, T-cell proliferation, and IFN-ɣ production, hereby inhibiting antitumor response mediated by the immune system [30–32]. Moreover, TGF-β is known to inhibit IL-2-dependent T-cell proliferation as well as production of proinflammatory cytokines and performance of cytolytic functions by effector cells [33–35]. Suggesting its involvement in chronic inflammatory liver disease and contribution to hepatocarcinogenesis, levels of the immunoregulatory cytokine IL-10 and TGF-β have been reported to be elevated in patients with chronic liver disease and related to disease progression and patients' survival [30, 36, 37].

Immune Cells in HCC Microenvironment

Leukocytes are one of the main drivers in chronic inflammation. They are highly enriched in both the precancerous state of liver cirrhosis and in malignant tissue of HCC. Indeed, liver carcinoma is characterized by an immunogenic microenvironment, consisting of high amounts of lymphocytes, including NK cells, NKT cells, B cells, and T cells [38]. T-cell exhaustion due to chronic inflammation hereby shapes an immunogenic microenvironment that is characterized by an enhanced immunotolerance. Thus, the endogenous antitumor function of cytotoxic lymphocytes can be restored by antigen-presenting cells, which are typically reduced in the HCC microenvironment [39]. Indeed, decreased activity of NK cells, one of the most important antigen-presenting cells, correlates with an increased incidence of HCC in patients with liver cirrhosis [40]. Moreover, infiltration and density of T cells in human HCCs correlate with better patient prognosis, whereas tumor-infiltrating B cells reduce tumor viability [41].

Macrophages perpetuate chronic inflammation following liver injury and promote fibrogenesis via HSC activation. This therefore represents a significant component of HCC microenvironment. Of note, tumor-associated macrophages (TAMs) are considered to promote tumor development and favor angiogenesis and tumor cell migration [42, 43]. Moreover, TAMs may stimulate tumor growth by suppression of the adaptive immune system. They express high levels of cell death-ligand 1 (PD-L1), thereby suppressing the antitumor cytotoxic T-cell responses [44]. TAMs provide cytokines and growth factors that enhance tumor cell proliferation and NF-κB-mediated protection from cancer cell apoptosis and angiogenesis [45]. Accordingly, TAM infiltration correlates with HCC progression and poor survival [46, 47].

Dendritic cells (DCs) are a heterogeneous cell population and one of the most powerful antigen-presenting cells which regulate the primary immune response and the immune homeostasis in the liver [48]. By forming a bridge between the innate and the adaptive immune system [49], DCs are regarded as key players in immune

regulation [50, 51]. An impaired DC function has frequently been suggested as an important factor contributing to an immunosuppressive microenvironment in chronic liver disease, which is favoring tumor development. Accordingly, several studies report lower DC numbers in both the peripheral blood and liver tissue of patients with HCC [52, 53]. A reduced IL-12 secretion by DCs is hereby attributed to an attenuated stimulation of T cells [54]. Moreover, DC inhibition and its effects on downstream effector cells have further been identified as immune escape mechanisms of HCC [55, 56].

Stromal Cells Participate in HCC Development and Progression

Liver cirrhosis is one of the main risk factors for hepatocarcinogenesis and therefore regarded as a precancerous liver state [57]. Thus, the lifetime risk of HCC development in patients with advanced liver cirrhosis is approximately 30%, and 80–90% of HCCs evolve in cirrhotic liver tissue [58, 59]. Considering HSCs as the most important progenitor cells of myofibroblasts that account for enhanced production of the extracellular matrix in liver fibrosis and liver cirrhosis, HSC-derived myofibroblasts are the main components of the hepatic precancerous microenvironment as well as the HCC tumor environment. Indeed, differentiation of HSCs from pericyte-like cells to collagen-producing myofibroblasts provides 85–95% of the myofibroblasts in liver fibrosis and liver cirrhosis, independent of the underlying trigger [15]. Hence, together with bone marrow (BM)-derived fibroblasts and portal fibroblasts (PF), HSC-derived myofibroblasts compose the stromal population of cancer-associated myofibroblasts (CAFs) that contribute actively to HCC development and progression [60]. Of note, CAFs show a markedly altered phenotype compared to normal fibroblasts [61, 62]. Normal fibroblasts may suppress tumor growth by contact inhibition [62], whereas CAFs promote an immune-tolerant tumor environment by interaction with monocytes and lymphocytes [63]. Indeed, CAFs inhibit lymphocyte tumor infiltration, increase the activity of immunosuppressive regulatory T cells, and induce apoptosis in monocytes [64, 65]. Furthermore, CAFs were reported to impair antitumor functions of T cells via activation of neutrophils [66]. CAFs may further promote hepatocarcinogenesis by downregulation of tumor-suppressive microRNAs [67, 68]. CAF activity has also been associated with tumor angiogenesis. CAFs have been shown to secrete vascular endothelial growth factor (VEGF) and angiopoietin 1 or 2 [69–71]. The cross talk between CAFs and cancer cells is crucial for HCC biology. The secretion of laminin 5 (LAMA5) [72] and IL-1β [73] by CAFs has been shown to promote HCC migration, and on the other hand, highly metastatic HCC cells were found to be able to convert normal fibroblasts to CAFs, which in turn promote cancer progression by secretion of proinflammatory cytokines [74]. Several studies further suggest an association of CAFs and CSCs that are thought to promote tumor development and to mediate therapeutic resistance. CAFs have been reported to recruit CSCs and to

drive their self-renewal [75, 76]. Moreover, CAFs have been observed to increase expression of keratin 19 by paracrine interactions [77], a marker for hepatic stem cells that has been observed to be correlated with poor prognosis [78]. In summary, CAFs are key drivers in hepatic carcinogenesis by increasing angiogenesis, inflammation, and proliferation and attenuating immune surveillance [60] (Fig. 15.2). CAFs correlate with HCC tumor stage and progression, tumor recurrence after surgery, as well as overall prognosis [79–81].

Lymphatic vessels function as a tissue drainage and immunological control system. They are highly enriched in the liver, carrying approximately 25–50% of the thoracic duct's lymph flow [82]. For a long time, lymphatic vessels were considered to affect carcinogenesis only by providing the structural pathway for metastatic spread of tumor cells. However, recent observations indicate a functional role of the lymphatic endothelium also in the hepatocytes' immunogenic microenvironment, which is affecting the development of chronic liver disease and hepatocarcinogenesis [83]. Thus, lymphatic endothelial cells (LECs) guide immune cell migration by lining the inner surface of lymphatic capillaries and regulate the expression of adhesion molecules and cytokines [84, 85]. Moreover, by secretion of immunosuppressive cytokines (i.e., TGF-β) and the overexpression of co-inhibitory checkpoint

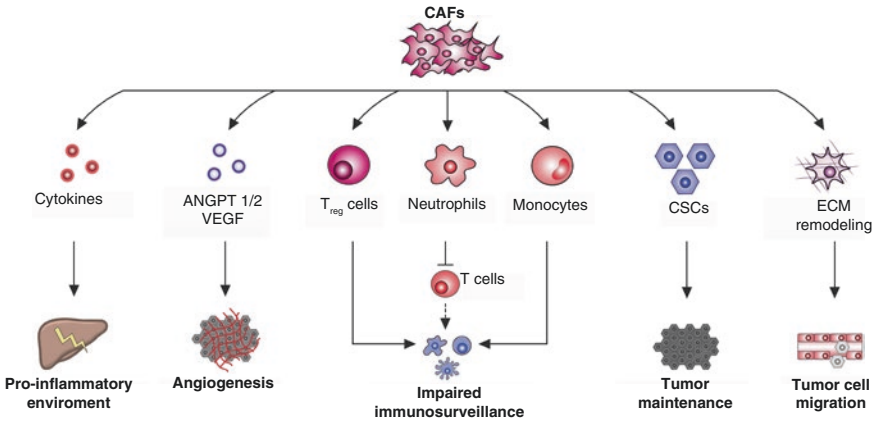

Fig. 15.2 Cancer-associated fibroblasts (CAFs) characterize the stromal tumor microenvironment and promote hepatocarcinogenesis, tumor progression and treatment resistance. Tumor microenvironment in HCC is predominantly characterized by cancer-associated fibroblasts (CAFs) that contribute actively to tumor development, progression and metastatic spread. Interacting with the immune cells and secreting angiogenic factors, these cells reduce immune surveillance and drive tumor angiogenesis. Moreover, CAFs promote cancer cell proliferation by paracrine interactions as well as production of prooncogenic cytokines (e.g. TGF-β). CAFs are also reported to recruit cancer stem cells, hereby affecting tumor maintenance, heterogeneity and treatment resistance. Finally, CAFs are responsible for the alteration of liver extracellular matrix by production and secretion of Laminin 5 and Integrin β1 that further promote HCC cell invasion and migration

proteins (i.e., PD-L1), LECs suppress a maturation and proliferation of circulating immune cells [84–86]. LECs further mediate $CD4^+$ and $CD8^+$ T-cell tolerance by expression of self-antigens in the presence of inhibitory ligands [87].

Lymphangiogenesis is increased in liver fibrosis and cirrhosis and positively correlate with portal venous pressure and disease severity [88–90]. The enhanced interstitial flow and increased number of LECs is accompanied by increased cytokine production and immune cell recruitment to the inflammatory environment present in almost all chronic liver diseases [91]. The primarily immunosuppressive functions of LECs hereby contribute to an immunotolerant microenvironment favoring HCC development [83, 92]. Moreover, expression of chemokines by LECs may facilitate lymphogenic metastatic tumor spread [84]. Vascular endothelial growth factor C (VEGF-C) is an important stimulator of LEC growth and lymphangiogenesis. VEGF-C is enhanced in liver cirrhosis and HCC, and its expression in HCCs correlates with metastasis and poor patients' outcome [93, 94].

Epithelial-to-Mesenchymal Transition in HCC

Epithelial-to-mesenchymal transition (EMT) describes a reversible process, by which epithelial cell types gradually develop mesenchymal characteristics leading to higher motility and invasive properties that are essential in embryogenic development and wound healing but also implicated in hepatic fibrogenesis and carcinogenesis [95, 96]. Thus, while epithelial cells are characterized by polarity and stable morphology, mesenchymal cells lack polarity, show a loose arrangement, and exhibit the capacity of migration [97]. EMT can be divided in three different biological subtypes [98]. While type 1 EMT determines embryonal development and organogenesis, types 2 and 3 EMT affect liver disease progression and can be activated by several proinflammatory cytokines and growth factors present in the inflammatory state of the liver [99].

Type 2 EMT occurs in response to cell injury as a mechanism of tissue repair and may cause fibrosis due to generation of collagen-producing fibroblasts. TGF-β, a cytokine increased under condition of chronic inflammation, has been shown to be one of the strongest activators of type 2 EMT that can affect hepatocytes, cholangiocytes, and hepatic stellate cells (HSC) [100]. Quiescent HSCs, the most frequent progenitor cells of collagen-producing fibroblasts [15], are actually regarded as transitional cells that have undergone partial EMT from epithelial cells and may complete transition upon inflammatory signals [101]. Hence, EMT is regarded as one of the most important promoters of liver fibrogenesis in response to chronic inflammation [101].

Type 3 EMT may occur due to genetic and epigenetic changes during malignant transformation of epithelial cells and is implicated in HCC growth and progression [3]. Cells generated by type 3 EMT differ significantly from types 1 and 2 EMT cells and develop properties of invasion and migration as well as escape from apop-

tosis. Weakened or loss of E-cadherin expression, characteristic for development of the mesenchymal unpolarized phenotype, could be revealed in 58% of human HCC patients and correlated with the presence of metastases and patients' survival [102]. Besides proinflammatory cytokines and growth factors, several studies further indicate induction of type 3 EMT by core proteins of HCV itself [103]. Given not only the correlation of EMT with tumor stage but also response to therapy [104], therapeutic targeting of molecular key players in EMT is highly clinically relevant.

Clinical Perspectives

Considering the implication of stromal and immunogenic cell compounds in HCC development and progression, medical treatments targeting these factors represent promising tools for future medical treatment of advanced HCC. Presently, sorafenib, an oral multikinase inhibitor targeting vascular endothelial growth factor receptor (VEGFR-2/VEGFR-3) and platelet-derived growth factor receptor (PDGFR), produced by the stromal HCC microenvironment already represents the standard of care treatment for patients with advanced HCC [105]. Lenvatinib, another tyrosine kinase inhibitor with multiple targets, has recently been revealed to be noninferior compared to sorafenib according to the REFLECT trial and has lately been approved by the FDA as first-line treatment for unresectable HCC [106]. Moreover, recently therapeutic strategies targeting the immunogenic tumor microenvironment have been demonstrated to be effective as systemic therapy for several cancer types. Consequently, drugs targeting exhausted lymphocytes expressing PD1 and infiltrating the tumor are able to activate T-cell-driven immune response against cancer cells and were approved for melanoma and non-small cell lung cancer treatment [107, 108]. Preliminary results from open-label trials of these drugs in HCC treatment are encouraging. Indeed, nivolumab and pembrolizumab, anti-PD1 monoclonal antibodies, have been demonstrated to be more effective than placebo in patients with advanced unresectable HCC previously treated with sorafenib [109, 110]. For that reason, these compounds were recently approved by FDA as a second-line treatment for advanced HCC. Moreover, currently several randomized controlled trials investigate the effects of other drugs targeting the HCC immunogenic and stromal microenvironment. Thus, aiming to activate tumor-targeting cytotoxic T lymphocytes, a growing number of studies recently worked on ex vivo tumor-antigen-loaded dendritic cells as an approach of cancer immunotherapy by DC vaccination [111–113]. Several other studies are focused on immunotherapy targeting TAMs, aiming to decrease TAM population present in the HCC by elimination, blocking recruitment, or functional reprogramming of TAM polarization [43]. The results of current ongoing clinical studies are expected in the next few years and may revolutionize future HCC medical treatment.

References

1. Jenne CN, Kubes P. Immune surveillance by the liver. Nat Immunol. 2013;14:996. https://doi.org/10.1038/ni.2691. https://www.nature.com/articles/ni.2691#supplementary-information.
2. Marrone G, Shah VH, Gracia-Sancho J. Sinusoidal communication in liver fibrosis and regeneration. J Hepatol. 2016;65(3):608–17. https://doi.org/10.1016/j.jhep.2016.04.018.
3. van Zijl F, Zulehner G, Petz M, Schneller D, Kornauth C, Hau M, et al. Epithelial-mesenchymal transition in hepatocellular carcinoma. Future Oncol. 2009;5(8):1169–79. https://doi.org/10.2217/fon.09.91.
4. Nishida N, Kudo M. Oncogenic signal and tumor microenvironment in hepatocellular carcinoma. Oncology. 2017;93(Suppl 1):160–4. https://doi.org/10.1159/000481246.
5. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med. 2008;359(19):1995–2004. https://doi.org/10.1056/NEJMoa0804525.
6. Caja L, Dituri F, Mancarella S, Caballero-Diaz D, Moustakas A, Giannelli G, et al. TGF-beta and the tissue microenvironment: relevance in fibrosis and cancer. Int J Mol Sci. 2018;19(5) https://doi.org/10.3390/ijms19051294.
7. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. Nat Immunol. 2018;19(3):222–32. https://doi.org/10.1038/s41590-018-0044-z.
8. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol. 2016;13(3):267–76. https://doi.org/10.1038/cmi.2016.3.
9. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2016;2:16018. https://doi.org/10.1038/nrdp.2016.18.
10. Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol. 2014;14(3):181–94. https://doi.org/10.1038/nri3623.
11. Mossanen JC, Krenkel O, Ergen C, Govaere O, Liepelt A, Puengel T, et al. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatology. 2016;64(5):1667–82. https://doi.org/10.1002/hep.28682.
12. Lemmers A, Moreno C, Gustot T, Marechal R, Degre D, Demetter P, et al. The interleukin-17 pathway is involved in human alcoholic liver disease. Hepatology. 2009;49(2):646–57. https://doi.org/10.1002/hep.22680.
13. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology. 2012;143(3):765–76 e3. https://doi.org/10.1053/j.gastro.2012.05.049.
14. Tu T, Calabro SR, Lee A, Maczurek AE, Budzinska MA, Warner FJ, et al. Hepatocytes in liver injury: victim, bystander, or accomplice in progressive fibrosis? J Gastroenterol Hepatol. 2015;30(12):1696–704. https://doi.org/10.1111/jgh.13065.
15. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun. 2013;4:2823. https://doi.org/10.1038/ncomms3823.
16. Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell. 2005;121(7):977–90. https://doi.org/10.1016/j.cell.2005.04.014.
17. Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell. 1994;77(1):63–71.
18. Mackey-Lawrence NM, Petri WA Jr. Leptin and mucosal immunity. Mucosal Immunol. 2012;5(5):472–9. https://doi.org/10.1038/mi.2012.40.

19. McCartney EM, Helbig KJ, Narayana SK, Eyre NS, Aloia AL, Beard MR. Signal transducer and activator of transcription 3 is a proviral host factor for hepatitis C virus. Hepatology. 2013;58(5):1558–68. https://doi.org/10.1002/hep.26496.
20. Haybaeck J, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, et al. A lymphotoxin-driven pathway to hepatocellular carcinoma. Cancer Cell. 2009;16(4):295–308. https://doi.org/10.1016/j.ccr.2009.08.021.
21. Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. Cancer Cell. 2014;26(4):549–64. https://doi.org/10.1016/j.ccell.2014.09.003.
22. Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature. 2011;479(7374):547–51. https://doi.org/10.1038/nature10599.
23. Ma C, Kesarwala AH, Eggert T, Medina-Echeverz J, Kleiner DE, Jin P, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature. 2016;531(7593):253–7. https://doi.org/10.1038/nature16969.
24. van der Windt DJ, Sud V, Zhang H, Varley PR, Goswami J, Yazdani HO, et al. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. Hepatology. 2018;68(4):1347–60. https://doi.org/10.1002/hep.29914.
25. Jiang R, Tang J, Chen Y, Deng L, Ji J, Xie Y, et al. The long noncoding RNA lnc-EGFR stimulates T-regulatory cells differentiation thus promoting hepatocellular carcinoma immune evasion. Nat Commun. 2017;8:15129. https://doi.org/10.1038/ncomms15129.
26. Li K, Liu H, Guo T. Th17/Treg imbalance is an indicator of liver cirrhosis process and a risk factor for HCC occurrence in HBV patients. Clin Res Hepatol Gastroenterol. 2017;41(4):399–407. https://doi.org/10.1016/j.clinre.2016.12.004.
27. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med. 2000;192(2):295–302.
28. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999;11(2):141–51.
29. Carter L, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR, et al. PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. Eur J Immunol. 2002;32(3):634–43. https://doi.org/10.1002/1521-4141(200203)32:3<634::AID-IMMU634>3.0.CO;2-9.
30. Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol. 1995;22(2):226–9.
31. Hattori E, Okumoto K, Adachi T, Takeda T, Ito J, Sugahara K, et al. Possible contribution of circulating interleukin-10 (IL-10) to anti-tumor immunity and prognosis in patients with unresectable hepatocellular carcinoma. Hepatol Res. 2003;27(4):309–14.
32. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765. https://doi.org/10.1146/annurev.immunol.19.1.683.
33. Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, et al. Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med. 1986;163(5):1037–50.
34. Espevik T, Waage A, Faxvaag A, Shalaby MR. Regulation of interleukin-2 and interleukin-6 production from T-cells: involvement of interleukin-1 beta and transforming growth factor-beta. Cell Immunol. 1990;126(1):47–56.
35. Smyth MJ, Strobl SL, Young HA, Ortaldo JR, Ochoa AC. Regulation of lymphokine-activated killer activity and pore-forming protein gene expression in human peripheral blood CD8+ T lymphocytes. Inhibition by transforming growth factor-beta. J Immunol. 1991;146(10):3289–97.

36. Othman MS, Aref AM, Mohamed AA, Ibrahim WA. Serum levels of Interleukin-6 and Interleukin-10 as biomarkers for hepatocellular carcinoma in Egyptian patients. ISRN Hepatol. 2013;2013:412317. https://doi.org/10.1155/2013/412317.
37. El-Emshaty HM, Nasif WA, Mohamed IE. Serum cytokine of IL-10 and IL-12 in chronic liver disease: the immune and inflammatory response. Dis Markers. 2015;2015:707254. https://doi.org/10.1155/2015/707254.
38. Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10(11):753–66. https://doi.org/10.1038/nri2858.
39. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1–10. https://doi.org/10.1016/j.immuni.2013.07.012.
40. Nakajima T, Mizushima N, Kanai K. Relationship between natural killer activity and development of hepatocellular carcinoma in patients with cirrhosis of the liver. Jpn J Clin Oncol. 1987;17(4):327–32.
41. Garnelo M, Tan A, Her Z, Yeong J, Lim CJ, Chen J, et al. Interaction between tumour-infiltrating B cells and T cells controls the progression of hepatocellular carcinoma. Gut. 2017;66(2):342–51. https://doi.org/10.1136/gutjnl-2015-310814.
42. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;124(2):263–6. https://doi.org/10.1016/j.cell.2006.01.007.
43. Degroote H, Van Dierendonck A, Geerts A, Van Vlierberghe H, Devisscher L. Preclinical and clinical therapeutic strategies affecting tumor-associated macrophages in hepatocellular carcinoma. J Immunol Res. 2018;2018:7819520. https://doi.org/10.1155/2018/7819520.
44. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. Cancer Res. 2009;69(20):8067–75. https://doi.org/10.1158/0008-5472.CAN-09-0901.
45. Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol. 2017;66(6):1300–12. https://doi.org/10.1016/j.jhep.2017.02.026.
46. Yeung OW, Lo CM, Ling CC, Qi X, Geng W, Li CX, et al. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. J Hepatol. 2015;62(3):607–16. https://doi.org/10.1016/j.jhep.2014.10.029.
47. Ding T, Xu J, Wang F, Shi M, Zhang Y, Li SP, et al. High tumor-infiltrating macrophage density predicts poor prognosis in patients with primary hepatocellular carcinoma after resection. Hum Pathol. 2009;40(3):381–9. https://doi.org/10.1016/j.humpath.2008.08.011.
48. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673):245–52. https://doi.org/10.1038/32588.
49. Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol. 2006;311:17–58.
50. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, et al. Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res. 2000;6(5):1755–66.
51. Steinman RM. Lasker Basic Medical Research Award. Dendritic cells: versatile controllers of the immune system. Nat Med. 2007;13(10):1155–9. https://doi.org/10.1038/nm1643.
52. Kakumu S, Ito S, Ishikawa T, Mita Y, Tagaya T, Fukuzawa Y, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. J Gastroenterol Hepatol. 2000;15(4):431–6.
53. Chen S, Akbar SM, Tanimoto K, Ninomiya T, Iuchi H, Michitaka K, et al. Absence of CD83-positive mature and activated dendritic cells at cancer nodules from patients with hepatocellular carcinoma: relevance to hepatocarcinogenesis. Cancer Lett. 2000;148(1):49–57.
54. Ormandy LA, Farber A, Cantz T, Petrykowska S, Wedemeyer H, Horning M, et al. Direct ex vivo analysis of dendritic cells in patients with hepatocellular carcinoma. World J Gastroenterol. 2006;12(20):3275–82.
55. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med. 1996;2(10):1096–103.
56. Beckebaum S, Zhang X, Chen X, Yu Z, Frilling A, Dworacki G, et al. Increased levels of interleukin-10 in serum from patients with hepatocellular carcinoma correlate with profound

numerical deficiencies and immature phenotype of circulating dendritic cell subsets. Clin Cancer Res. 2004;10(21):7260–9. https://doi.org/10.1158/1078-0432.CCR-04-0872.
57. Maier KP. Cirrhosis of the liver as a precancerous condition. Praxis (Bern 1994). 1998;87(44):1462–5.
58. Singal AG, El-Serag HB. Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. Clin Gastroenterol Hepatol. 2015;13(12):2140–51. https://doi.org/10.1016/j.cgh.2015.08.014.
59. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27. https://doi.org/10.1056/NEJMra1001683.
60. Affo S, Yu LX, Schwabe RF. The role of cancer-associated fibroblasts and fibrosis in liver cancer. Annu Rev Pathol. 2017;12:153–86. https://doi.org/10.1146/annurev-pathol-052016-100322.
61. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.
62. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011;17(3):320–9. https://doi.org/10.1038/nm.2328.
63. Ji J, Eggert T, Budhu A, Forgues M, Takai A, Dang H, et al. Hepatic stellate cell and monocyte interaction contributes to poor prognosis in hepatocellular carcinoma. Hepatology. 2015;62(2):481–95. https://doi.org/10.1002/hep.27822.
64. Zhao W, Su W, Kuang P, Zhang L, Liu J, Yin Z, et al. The role of hepatic stellate cells in the regulation of T-cell function and the promotion of hepatocellular carcinoma. Int J Oncol. 2012;41(2):457–64. https://doi.org/10.3892/ijo.2012.1497.
65. Zhao W, Zhang L, Yin Z, Su W, Ren G, Zhou C, et al. Activated hepatic stellate cells promote hepatocellular carcinoma development in immunocompetent mice. Int J Cancer. 2011;129(11):2651–61. https://doi.org/10.1002/ijc.25920.
66. Cheng Y, Li H, Deng Y, Tai Y, Zeng K, Zhang Y, et al. Cancer-associated fibroblasts induce PDL1+ neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma. Cell Death Dis. 2018;9(4):422. https://doi.org/10.1038/s41419-018-0458-4.
67. Zhang Z, Li X, Sun W, Yue S, Yang J, Li J, et al. Loss of exosomal miR-320a from cancer-associated fibroblasts contributes to HCC proliferation and metastasis. Cancer Lett. 2017;397:33–42. https://doi.org/10.1016/j.canlet.2017.03.004.
68. Wang F, Li L, Piontek K, Sakaguchi M, Selaru FM. Exosome miR-335 as a novel therapeutic strategy in hepatocellular carcinoma. Hepatology. 2018;67(3):940–54. https://doi.org/10.1002/hep.29586.
69. Torimura T, Ueno T, Kin M, Harada R, Taniguchi E, Nakamura T, et al. Overexpression of angiopoietin-1 and angiopoietin-2 in hepatocellular carcinoma. J Hepatol. 2004;40(5):799–807. https://doi.org/10.1016/j.jhep.2004.01.027.
70. Taura K, De Minicis S, Seki E, Hatano E, Iwaisako K, Osterreicher CH, et al. Hepatic stellate cells secrete angiopoietin 1 that induces angiogenesis in liver fibrosis. Gastroenterology. 2008;135(5):1729–38. https://doi.org/10.1053/j.gastro.2008.07.065.
71. Lin N, Chen Z, Lu Y, Li Y, Hu K, Xu R. Role of activated hepatic stellate cells in proliferation and metastasis of hepatocellular carcinoma. Hepatol Res. 2015;45(3):326–36. https://doi.org/10.1111/hepr.12356.
72. Santamato A, Fransvea E, Dituri F, Caligiuri A, Quaranta M, Niimi T, et al. Hepatic stellate cells stimulate HCC cell migration via laminin-5 production. Clin Sci (Lond). 2011;121(4):159–68. https://doi.org/10.1042/CS20110002.
73. Okabe H, Beppu T, Ueda M, Hayashi H, Ishiko T, Masuda T, et al. Identification of CXCL5/ENA-78 as a factor involved in the interaction between cholangiocarcinoma cells and cancer-associated fibroblasts. Int J Cancer. 2012;131(10):2234–41. https://doi.org/10.1002/ijc.27496.
74. Fang T, Lv H, Lv G, Li T, Wang C, Han Q, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018;9(1):191. https://doi.org/10.1038/s41467-017-02583-0.

75. Jiang J, Ye F, Yang X, Zong C, Gao L, Yang Y, et al. Peri-tumor associated fibroblasts promote intrahepatic metastasis of hepatocellular carcinoma by recruiting cancer stem cells. Cancer Lett. 2017;404:19–28. https://doi.org/10.1016/j.canlet.2017.07.006.
76. Liu C, Liu L, Chen X, Cheng J, Zhang H, Zhang C, et al. LSD1 stimulates cancer-associated fibroblasts to drive Notch3-dependent self-renewal of liver cancer stem-like cells. Cancer Res. 2018;78(4):938–49. https://doi.org/10.1158/0008-5472.CAN-17-1236.
77. Rhee H, Kim HY, Choi JH, Woo HG, Yoo JE, Nahm JH, et al. Keratin 19 expression in hepatocellular carcinoma is regulated by fibroblast-derived HGF via a MET-ERK1/2-AP1 and SP1 Axis. Cancer Res. 2018;78(7):1619–31. https://doi.org/10.1158/0008-5472.CAN-17-0988.
78. Kim H, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, et al. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. Hepatology. 2011;54(5):1707–17. https://doi.org/10.1002/hep.24559.
79. Coulouarn C, Corlu A, Glaise D, Guenon I, Thorgeirsson SS, Clement B. Hepatocyte-stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. Cancer Res. 2012;72(10):2533–42. https://doi.org/10.1158/0008-5472.CAN-11-3317.
80. Ju MJ, Qiu SJ, Fan J, Xiao YS, Gao Q, Zhou J, et al. Peritumoral activated hepatic stellate cells predict poor clinical outcome in hepatocellular carcinoma after curative resection. Am J Clin Pathol. 2009;131(4):498–510. https://doi.org/10.1309/AJCP86PPBNGOHNNL.
81. Zhang DY, Goossens N, Guo J, Tsai MC, Chou HI, Altunkaynak C, et al. A hepatic stellate cell gene expression signature associated with outcomes in hepatitis C cirrhosis and hepatocellular carcinoma after curative resection. Gut. 2016;65(10):1754–64. https://doi.org/10.1136/gutjnl-2015-309655.
82. Pupulim LF, Vilgrain V, Ronot M, Becker CD, Breguet R, Terraz S. Hepatic lymphatics: anatomy and related diseases. Abdom Imaging. 2015;40(6):1997–2011. https://doi.org/10.1007/s00261-015-0350-y.
83. Lund AW, Wagner M, Fankhauser M, Steinskog ES, Broggi MA, Spranger S, et al. Lymphatic vessels regulate immune microenvironments in human and murine melanoma. J Clin Invest. 2016;126(9):3389–402. https://doi.org/10.1172/JCI79434.
84. Swartz MA. Immunomodulatory roles of lymphatic vessels in cancer progression. Cancer Immunol Res. 2014;2(8):701–7. https://doi.org/10.1158/2326-6066.CIR-14-0115.
85. Lukacs-Kornek V, Malhotra D, Fletcher AL, Acton SE, Elpek KG, Tayalia P, et al. Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. Nat Immunol. 2011;12(11):1096–104. https://doi.org/10.1038/ni.2112.
86. Fletcher AL, Lukacs-Kornek V, Reynoso ED, Pinner SE, Bellemare-Pelletier A, Curry MS, et al. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. J Exp Med. 2010;207(4):689–97. https://doi.org/10.1084/jem.20092642.
87. Rouhani SJ, Eccles JD, Riccardi P, Peske JD, Tewalt EF, Cohen JN, et al. Roles of lymphatic endothelial cells expressing peripheral tissue antigens in CD4 T-cell tolerance induction. Nat Commun. 2015;6:6771. https://doi.org/10.1038/ncomms7771.
88. Vollmar B, Wolf B, Siegmund S, Katsen AD, Menger MD. Lymph vessel expansion and function in the development of hepatic fibrosis and cirrhosis. Am J Pathol. 1997;151(1):169–75.
89. Yamauchi Y, Michitaka K, Onji M. Morphometric analysis of lymphatic and blood vessels in human chronic viral liver diseases. Am J Pathol. 1998;153(4):1131–7. https://doi.org/10.1016/S0002-9440(10)65657-X.
90. Yokomori H, Oda M, Kaneko F, Kawachi S, Tanabe M, Yoshimura K, et al. Lymphatic marker podoplanin/D2-40 in human advanced cirrhotic liver--re-evaluations of microlymphatic abnormalities. BMC Gastroenterol. 2010;10:131. https://doi.org/10.1186/1471-230X-10-131.
91. Limatola E, Filosa S. Exogenous vitellogenesis and micropinocytosis in the lizard, Podarcis sicula, treated with follicle-stimulating hormone. Gen Comp Endocrinol. 1989;75(2):165–76.

92. Shields JD, Kourtis IC, Tomei AA, Roberts JM, Swartz MA. Induction of lymphoid-like stroma and immune escape by tumors that express the chemokine CCL21. Science. 2010;328(5979):749–52. https://doi.org/10.1126/science.1185837.
93. Xiang Z, Zeng Z, Tang Z, Fan J, Sun H, Wu W, et al. Increased expression of vascular endothelial growth factor-C and nuclear CXCR4 in hepatocellular carcinoma is correlated with lymph node metastasis and poor outcome. Cancer J. 2009;15(6):519–25. https://doi.org/10.1097/PPO.0b013e3181c6aa6b.
94. Yamaguchi R, Yano H, Nakashima O, Akiba J, Nishida N, Kurogi M, et al. Expression of vascular endothelial growth factor-C in human hepatocellular carcinoma. J Gastroenterol Hepatol. 2006;21(1 Pt 1):152–60. https://doi.org/10.1111/j.1440-1746.2005.04217.x.
95. Nieto MA, Huang RY, Jackson RA, Thiery JP. Emt: 2016. Cell. 2016;166(1):21–45. https://doi.org/10.1016/j.cell.2016.06.028.
96. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol. 2018; https://doi.org/10.1038/s41580-018-0080-4.
97. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009;9(4):265–73. https://doi.org/10.1038/nrc2620.
98. Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest. 2009;119(6):1438–49. https://doi.org/10.1172/JCI38019.
99. Yan L, Xu F, Dai CL. Relationship between epithelial-to-mesenchymal transition and the inflammatory microenvironment of hepatocellular carcinoma. J Exp Clin Cancer Res. 2018;37(1):203. https://doi.org/10.1186/s13046-018-0887-z.
100. Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene. 2005;24(37):5764–74. https://doi.org/10.1038/sj.onc.1208927.
101. Choi SS, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. Hepatology. 2009;50(6):2007–13. https://doi.org/10.1002/hep.23196.
102. Zhai B, Yan HX, Liu SQ, Chen L, Wu MC, Wang HY. Reduced expression of E-cadherin/catenin complex in hepatocellular carcinomas. World J Gastroenterol. 2008;14(37):5665–73.
103. Battaglia S, Benzoubir N, Nobilet S, Charneau P, Samuel D, Zignego AL, et al. Liver cancer-derived hepatitis C virus core proteins shift TGF-beta responses from tumor suppression to epithelial-mesenchymal transition. PLoS One. 2009;4(2):e4355. https://doi.org/10.1371/journal.pone.0004355.
104. Fuchs BC, Fujii T, Dorfman JD, Goodwin JM, Zhu AX, Lanuti M, et al. Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. Cancer Res. 2008;68(7):2391–9. https://doi.org/10.1158/0008-5472.CAN-07-2460.
105. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90. https://doi.org/10.1056/NEJMoa0708857.
106. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. 2018;391(10126):1163–73. https://doi.org/10.1016/S0140-6736(18)30207-1.
107. Sui H, Ma N, Wang Y, Li H, Liu X, Su Y, et al. Anti-PD-1/PD-L1 therapy for non-small-cell lung cancer: toward personalized medicine and combination strategies. J Immunol Res. 2018;2018:6984948. https://doi.org/10.1155/2018/6984948.
108. Herzberg B, Fisher DE. Metastatic melanoma and immunotherapy. Clin Immunol. 2016;172:105–10. https://doi.org/10.1016/j.clim.2016.07.006.
109. Killock D. Immunotherapy: Nivolumab keeps HCC in check and opens avenues for checkmate. Nat Rev Clin Oncol. 2017;14(7):392. https://doi.org/10.1038/nrclinonc.2017.70.

110. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018;19(7):940–52. https://doi.org/10.1016/S1470-2045(18)30351-6.
111. Shang N, Figini M, Shangguan J, Wang B, Sun C, Pan L, et al. Dendritic cells based immunotherapy. Am J Cancer Res. 2017;7(10):2091–102.
112. Palmer DH, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, et al. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. Hepatology. 2009;49(1):124–32. https://doi.org/10.1002/hep.22626.
113. El Ansary M, Mogawer S, Elhamid SA, Alwakil S, Aboelkasem F, Sabaawy HE, et al. Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC. J Cancer Res Clin Oncol. 2013;139(1):39–48. https://doi.org/10.1007/s00432-012-1298-8.

Chapter 16
Experimental Models for Preclinical Research in Hepatocellular Carcinoma

Pedro Molina-Sánchez and Amaia Lujambio

Introduction

It is estimated that more than ¾ million people in the world are diagnosed with liver cancer every year [1], 85% of them presenting its most common form, hepatocellular carcinoma (HCC) [2]. HCC is described as a poor prognosis malignancy due to its difficult detection in early stages, when curative therapies are available, along with its strong metastatic capacity [3] and high frequency of recurrence [4]. Some of the major risk factors associated with HCC include viral hepatitis, alcohol abuse, obesity, and diabetes [5], which have all been on the raise in recent years. This increased incidence of risk factors has resulted in a dramatic rise in occurrence of

P. Molina-Sánchez
Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

A. Lujambio (✉)
Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Graduate School of Biomedical Sciences at Icahn School of Medicine at Mount Sinai, New York, NY, USA
e-mail: Amaia.Lujambio@mssm.edu

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_16

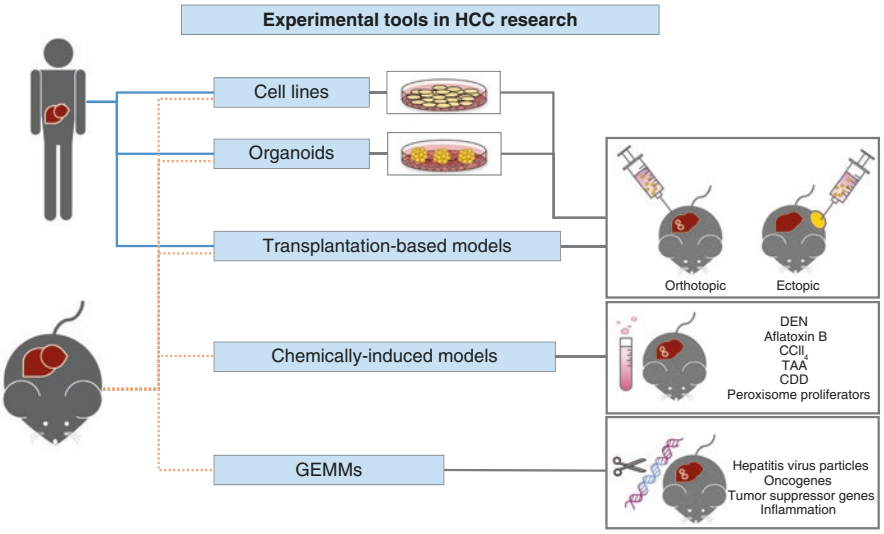

Fig. 16.1 Experimental models of hepatocellular carcinoma

HCC cases in the last years [6]. Unfortunately, unlike other tumor malignancies, HCC currently presents a limited number of available therapeutic approaches [2]. Therefore, it is essential to develop a comprehensive research plan in order to harness new diagnostic methods and efficient treatments to attack this disease. To this aim, numerous models have been established in the last decades, enabling scientists to study every biological aspect of human HCC (including malignant transformation, tumor progression and dissemination, and tumor microenvironment) and to evaluate tumor responses to novel treatments (Fig. 16.1). So far, due to their intrinsic properties, murine and human tumor-based models have been the most successful instruments for this purpose. Nevertheless, there is no model able to reproduce the entire nature and complexity of human disease, making model selection a critical step to successfully achieve the desired research objectives. In this chapter we compile some of the most widely used preclinical HCC models, outlining the characteristics that make them suitable to understand the different aspects of liver cancer.

In Vitro Models

HCC Cell Lines

Human and murine cell lines are routinely used in biomedical research laboratories for a better understanding of cancer biology. Their faithfulness is a matter of discussion since it is accepted that cultured cells present important limitations when

recapitulating the complexity of the original tumors. For example, cell lines are usually obtained from a limited tumor portion in a very specific time in tumor evolution, fail to reproduce the implications of growing in three dimensions (3D), and are isolated from its natural environment, which includes the host's immune response [7]. However, cell lines present certain unrivaled advantages for cancer research as they represent a homogenous population (providing consistent data), are effortlessly maintained/expanded, and can be manipulated easily [7]. There are dozens of cell lines established from HCCs, both of human and animal origins, that are currently available for research purposes. This wide variety of HCC cell lines constitutes a very valuable tool for researchers, but a large cell line catalogue can also complicate the selection of the appropriate line or lines for each research purpose. Initiatives such as the Cancer Cell Line Encyclopedia (CCLE), Cellosaurus (ExPASy-SIB), and other platforms (e.g., NCI-60) have emerged with the objective of collecting as much information as possible (gene expression profiles, genomic and chromosomal alterations, mutational landscape, etc.) to provide complete cellular databases to guide investigators.

The most commonly employed cancer cell line in liver research is HepG2. Established from a Caucasian adolescent, this cell line has been traditionally considered as a "pure" HCC cell line since it is not infected with hepatitis virus and presents intact characteristics associated to human neoplastic lesions, such as increased α-fetoprotein (AFP), α2-macroglobulin, and transferrin expression [8]. Nevertheless, the use of HepG2 cells has become a matter of controversy since some studies suggest that HepG2 cell line could have been originated from a more epithelial hepatoblastoma-like tumor [9]. Another very frequently used HCC cell line is Huh7. This cell line was obtained from a well-differentiated HCC tumor from a middle-aged Japanese patient [10], and it has been described to be an appropriate model to study the implications of hepatitis C virus (HCV) infection, in particular related to cancer initiation [11–13]. It is worth noting that Huh7 cell line presents a point mutation at codon 220 of tumor suppressor *TP53* which, contrary to most p53 mutations observed in HCC cell lines, results in increased levels of the protein [14]. This characteristic may be of interest when studying the functional consequences of different p53 mutations in liver carcinogenesis. Other cell lines such as HepaRG, BEL-7402, Hep3B, SKHep1, or SMMC-7721 have been traditionally used in HCC studies. However, it has been recently reported that some of them (including SMMC-7721, BEL-7402, or SKHep1) may be contaminated with cells of diverse origins, and it is recommended that these cell lines are not used for HCC studies [15].

Most human HCC cell lines can be obtained from different cell banks, such as the Japanese Collection of Research Bioresources (JCRB), the American Type Culture Collection (ATCC), or the Cell Bank of Chinese Academy of Science. Major applications of HCC cell lines include the study of cell proliferation control, immortality acquisition, and metastatic progression [16]. Furthermore, HCC cell lines are broadly used as tools for target discovery or in drug screens [17] and are essential in the development of in vivo xenograft models.

HCC Organoids

The idea to create 3D cell cultures and/or organoids was born many years ago in an effort to address some of the limitations of traditional cell cultures. But deficient technologies and lack of understanding of stem cell biology delayed their full development until recently. One of the most accepted descriptions for "organoid" was given by Fatehullah et al., being defined as "in vitro 3D cellular clusters derived exclusively from primary tissue, embryonic stem cells, or induced pluripotent stem cells, capable of self-renewal and self-organization, and exhibiting similar organ functionality as the tissue of origin" [18]. Due to their characteristics, organoids have been added to the repertoire of cancer research tools as they theoretically combine the benefits of both in vitro and in vivo models. For example, organoids can be propagated for a long time and amplified from a small tissue sample, can be adapted to high-throughput research approaches, and can keep cellular complexity and 3D structure. In the last few years, a wide variety of human and murine organoids have been established from different organs [19]. In the context of human liver, Tabeke et al. were pioneers by generating 3D aggregates of human pluripotent stem cell-derived hepatocytes in combination with endothelial and mesenchymal cells in a Matrigel matrix [20]. At the same time, Meritxell Huch and colleagues have optimized several protocols to grow adult human and mouse liver organoids [21–23]. These liver organoids exhibit equivalent genetic and histological characteristics to the tissue of origin and are amenable for genetic manipulation [21], which will enable performing functional experiments. Moreover, HCC patient-derived organoids have also been generated [24]. Exome sequencing analyses exhibit low mutation rates during HCC organoid expansion, and gene expression profiles show high correlation with the corresponding human HCCs [24]. In addition, it has been also demonstrated the ability of HCC organoids to grow in vivo when injected into mice, even displaying the ability to induce metastasis [24]. Although this technology is quite recent, the initial results suggest that HCC organoids could be very promising for liver cancer research. Thus, organoids could be used in drug screens and toxicology studies, enable precision medicine when directly derived from HCC patients, and be applied to transplantation strategies. Nonetheless, organoids still require several improvements as they are more expensive and time-consuming than regular cancer cell lines, are difficult to generate, and lack essential components of in vivo systems such as blood vessels or the immune compartment [18].

In Vivo Models

Transplantation-Based Models: Xenografts and Allografts

By definition, xenografting consists in the transplantation of a living entity (cell, tissue, organ, or system) from one species to another. In the HCC field, this method generally involves the transplantation of human HCC samples (tumor-derived cells

or tumor tissue) into mice, either in the liver (orthotopic xenograft) or under the skin (ectopic xenograft) [25]. These transplantation-based models emerged to take into consideration the role of tissue microenvironment in cancer progression, an aspect that is absent in the majority of in vitro methods. A fundamental issue to be considered when establishing xenografts is that the recipient must accept the "foreign" tissue so that it can survive and grow in the host organism. This implies the use of immune-deficient mice. Some of the most common mouse strains used for xenografting are *nu/nu* nude mice (deficient of T cells), severe combined immune-deficient (SCID) mice (lacking T and B cells), athymic nude mice (presenting *Foxn1* deletion and consequently abnormal thymus and defective T cells), nonobese diabetic/severe combined immune-deficient (NOD/SCID) mice, and the recombination-activating gene 2 (*Rag2*) knockout mice (unable to produce mature B and T lymphocytes) [26–28]. Xenograft models are largely designed to test new drugs or combination therapies, which may be administrated through oral gavage, intraperitoneal (IP), intravenous (IV), or intratumoral injection, the latter mimicking the clinically used transcatheter arterial chemoembolization (TACE).

The extent and growth pattern of the xenografts are affected by different parameters such as the size of the transplanted tumor tissue or the nature and number of the cells injected, requiring from 1 week to even more than 5 months to develop [29]. The most common and easiest way to establish HCC xenografts is through the injection of human tumor cell lines subcutaneously, generally placed on the flanks or on the back of the mouse. This is a suited strategy when studying cellular response to drugs as it provides a rapid growth model that can be easily tracked. However, cell line-derived xenografts lack the typical tumor cell diversity or heterogeneity, which can potentially lead to inaccurate experimental conclusions. Some representative examples of this method have been reviewed elsewhere [30]. The subcutaneous transplantation of human tumors into mice (known as patient-derived xenografts or PDXs) can overcome some of these limitations and provides a more reliable model of HCC. However, the establishment of PDXs is challenging as the engraftment success strongly depends on every tumor model. Both ectopic approaches, however, present an unnatural environment for tumor growth. In the orthotopic xenograft models, in contrast, tumor cells are implanted directly in the liver, which provides a more physiological context. The orthotopic model is more technically challenging than the ectopic as it requires surgical intervention, but it also facilitates tumor cell dissemination, enabling the study of metastatic progression [31].

One of the major disadvantages of the xenograft models is the impossibility to faithfully study the role of the immune system in tumor development, which limits the understanding of the contribution of immune cells to HCC origin, progression, and drug response. Similarly, xenografts are also inadequate to test drugs that activate the immune system. This is a major issue since immunotherapy is emerging as a key therapy for HCC treatment [32, 33]. Allograft models, in which tumor cells from one species are transplanted into animals from the same species, are a widely used alternative as they preserve an intact immune system. Allografts can be combined with genome engineering tools to more precisely dissect HCC. While allografts allow studying the interactions between immune cells and HCC cells, there are species-specific differences that are important to keep in mind when

reaching conclusions that may not completely apply to humans. Some relevant examples of liver cancer allografts and other transplantation-based models can be found elsewhere [25].

Chemically Induced Models

Undoubtedly, xenobiotic detoxification is one of the major functions of the liver. Humans are exposed during their lifetimes to a very broad spectrum of molecules in different doses and periods, many of them affecting liver homeostasis and inducing disease [34, 35]. The identification of hepatotoxic compounds and the full understanding of the underlying mechanisms of carcinogenesis is an ongoing public health goal. As a consequence, decades of liver investigation have already brought into light a considerable number of compounds with very defined roles in tumorigenesis and cancer progression [36]. Some of those compounds have been frequently tested in animals (mostly mice and rats) in order to reproduce human disease. These molecules are classified as genotoxics (inducing DNA damage) and promoters (accelerating tumor progression after malignant transformation). Chemically induced models are very valuable tools to understand human HCC as they reproduce the typical damage and healing episodes observed in the human setting. However, each hepatotoxin produces particular liver lesions according to its nature, its mechanisms of action, and other extrinsic factors such as administration route, animal strain, gender, age, dose, and treatment schedule. Some of the most relevant models of HCC based on the administration of chemical compounds are described below.

Diethylnitrosamine (DEN)

DEN or diethylnitrosamine ($C_4H_{10}N_2O$) is a member of the N-nitroso group of compounds and an extensively described carcinogenic molecule. The first time that DEN was described as a tumorigenic agent was precisely in a study to evaluate the oncogenic effect of this compound in the liver using experimental rats [37], although other members of this same family were already considered as potential carcinogens. Since then, DEN administration has been commonly used to induce tumorigenesis in rodents. There are two primary mechanisms underlying DEN tumorigenesis: its capacity to induce DNA adduct formation [38], which promotes genomic point mutations and consequently carcinogenesis (when affecting driver genes), and the stimulation of the cytochrome P450, increasing the production of reactive oxygen species (ROS) in the liver [39, 40]. The effect of DEN in mice is strongly affected by the animal strain (and therefore, the underlying genetic background). SWR, C57BL/6, or BALB/c mouse strains are considered more resistant to tumor formation by DEN than SM/J, FVB, CE/J, P/J, LP, or AKR/J strains, while CBA and C3H strains are highly sensitive to DEN-based liver carcinogenesis [41, 42].

The dose (ranging from 1 mg/kg to 100 mg/kg) and the number of administrations (single administration or multiple administrations over time), together with the gender, the weight, and the age of the animals, are important parameters that dramatically affect the final outcome of DEN treatment [41]. IP injection is the favorite route of administration since it enables a better control and accuracy of the dosage, although we can find studies with very diverse administration methods, such as oral gavage, drinking water, inhalation, and even intragastrical or intratracheal instillation. Regarding gender discrepancies, long-term administration of DEN induces HCC in 100% of male mice but only in approximately a third of females [43, 44], recapitulating the gender discrepancies observed in humans (men's incidence is 2.5–3 times higher than women's). DEN can therefore be used to better understand the role of gender in HCC development and suggests that the gender disparities observed in humans are not only attributed to disparate lifestyles but also to the opposite impact that estrogens and androgens have in hepatocarcinogenesis [43, 44]. In terms of genetic damage, recent studies based on whole exome sequencing analysis have provided a detailed mutational characterization of the DEN-induced murine tumors, showing that DEN treatment induces a mutational imprint affecting *Hras*, *Braf*, *Egfr*, and *Apc* but a higher burden of mutations compared to human HCCs [45]. A detailed review on DEN models in liver cancer is presented elsewhere [42].

DEN-induced liver tumor models present some significant limitations to be considered. DEN-based models are poorly reproducible, consequently requiring high numbers of animals [29]. In addition, metastases are not observed after DEN treatment, restricting its application to the study of primary tumors. Finally, it is worth noting that tumor formation can be relatively slow (taking up to 100 weeks) through most routes of administration and treatment regimens [29]. Regarding this last issue, there are a few chemical agents that do not induce malignant transformation of hepatocytes but accelerate tumorigenesis after DEN-mediated tumor initiation. In this respect, phenobarbital is a widely used partner of DEN. Phenobarbital is a barbiturate commonly used for treating epilepsy that shows an interesting HCC-promoting effect in rodents. The mechanisms by which phenobarbital enhances DEN tumorigenic activity are not totally understood, but they have been attributed to the induction of cytochrome P450 expression [46] and its role in promoting DNA hypermethylation of tumor suppressor genes (TSGs) [47]. The administration of phenobarbital not only results in more aggressive tumors in a significantly shorter time (from 12 to 40 weeks) but also stimulates the induction of metastasis enabling the study of this critical aspect of cancer progression.

Aflatoxin Exposure

Aflatoxins are mycotoxins produced by some members of the *Aspergillus* gender of fungi (mostly by *Aspergillus flavus* and *Aspergillus parasiticus* [48]). These fungi are commonly found as contaminants of diverse types of nuts and cereals (corn, wheat, rice, and other oil plants) when kept in a humid and warm environment and

represent a major HCC risk factor in countries with non-strict food control regulations [49]. Aflatoxin B1 is the most carcinogenic molecule within this family. When captured by liver cells, cytochrome P450 transforms it in its exo-eposide form, which is primarily responsible for DNA adduct formation [50]. Aflatoxin B1 mostly induces guanine to thymine transversion in genomic DNA and consequently increases the mutational load in hepatocytes, eventually resulting in malignant transformation [51]. Aflatoxin products have been intimately related with mutations in tumor suppressor *p53* (G:C to T:A transversion in 249ser codon) [52], which can be a potential mechanism of tumorigenesis. Nevertheless, single administration of 6 mg/kg of aflatoxin B1 into 1-week-old mice led to liver cancer development within 1 year, presenting features of human tumors but showing no compromising mutations in *p53*, suggesting that other mechanisms are involved, at least in mice [53]. HCC induction by aflatoxin treatment is very strain-dependent in mice, highlighting that different genetic variants affecting detoxification genes can significantly affect aflatoxin-mediated transformation and, consequently, liver cancer susceptibility [53]. Another interesting fact is that aflatoxin B and hepatitis B and C virus (HBV and HCV) infection could have a synergistic effect in HCC development [54, 55]. One potential explanation is that DNA mutations could appear as a result of ROS production in the chronic inflammatory context produced by the virus [55]. Accordingly, the model may be appropriate not only to identify the pathological mechanisms linked to aflatoxin exposure in humans but also to study the role of hepatitis infection on liver tumorigenesis.

Carbon Tetrachloride (CCl_4)

CCl_4 (carbon tetrachloride) is the most employed hepatotoxic molecule for modeling human liver disease due to its capacity to induce liver damage. In rodents, its administration through IP injection, inhaled, or in drinking water, leads to the production of trichloromethyl radicals after cytochrome P450-mediated transformation, which results in ROS production and inflammatory response induced by hepatic stellate cells (HSCs) and particularly by liver macrophages (Kupffer cells) [56]. CCl_4 does not induce direct mutagenesis in the hepatocytes but contributes to fibrosis after several rounds of injury and healing. Consequently, repeated CCl_4 treatment provokes massive hepatic fibrosis that eventually ends up in tumorigenesis. While this model is very interesting since it recapitulates the common steps that lead to HCC in humans, it can be rather slow, taking up to several months for tumor development depending on the mouse strain [57]. Combined treatment of CCl_4 with other hepatotoxic molecules can however solve this problem. This is the case of the two-staged DEN-CCl_4 model, based on a single IP injection of DEN (1 mg/kg) at 2 weeks of age and followed by repeated doses of CCl_4 (0.2 ml/kg, IP) [58]. In this model, the initial DEN administration produces genotoxic effects that are enhanced by CCl_4-induced fibrosis, increasing the incidence of tumor development in mice [58]. The molecular and genetic alterations

detected when following this protocol are similar to those observed in the human disease, which allows the study of the genetic alterations associated with severe liver fibrosis.

Thioacetamide (TAA)

Thioacetamide (TAA) was identified as a hepatotoxin in the mid-twentieth century, shortly after its introduction as a fungicide [59]. Similar to CCl_4, this organosulfur compound has been used routinely to induce fibrosis and cirrhosis in rodents (mostly in rats) due to its capacity to produce liver injury. TAA, while is not a direct genotoxic, can be administered on its own or in combination with other molecules (such as DEN) to induce HCC in mice. Despite being used for a long time, the mechanisms by which TAA leads to tumor formation are still unclear. One possibility is that TAA is transformed into its oxidized forms (TAA-S-oxide and TAA-S,S-oxide) by the FAD-containing and the cytochrome P450-depending monooxygenases, since it has not been described that TAA by itself produces toxic effects in hepatocytes. These secondary forms may be responsible for glutathione depletion and oxidative stress in the cells, causing liver damage. It has also been attributed to TAA the capacity to join covalently to essential cellular components such as lipids and proteins, impairing cellular homeostasis [60]. As in many chemically induced models, the chronic administration of this molecule in rodents causes repetitive cycles of injury and healing in the liver, eventually leading to malignant transformation. TAA has been administered to different rodent strains through very diverse routes, and in different doses, which determines liver injury grade. In most of the cases though, liver histology reported common human-like fibrosis appearance [61]. The most often administration routes selected are IP injection (in repeated administrations) or through drinking water (at a concentration of 0.02–0.05%), which can promote HCC initiation after at least 20 weeks of exposure [62]. Nevertheless, despite constant TAA exposure frequently resulting in malignant transformation in the liver, this model is barely used to study HCC nowadays.

Choline-Deficient Diet (CDD)

Choline is an essential nutrient metabolized by the liver. Its function in cell biology is heterogeneous since it participates in cell membrane signaling pathways as well as in lipid transportation or in the synthesis of neurotransmitters [63]. Low-choline diets have been shown to induce liver damage, starting with fat accumulation and ending with cirrhosis and liver cancer in the more severe cases [64]. The molecular mechanism for choline-induced liver injury is not totally understood, but it is possibly related to defects in lipoprotein production (mostly impaired hepatic very-low-density lipoprotein secretion), anomalous phospholipid metabolism, and/or mitochondrial dysfunction [64]. Choline-deficient diets (CDDs) have been

traditionally used as models of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (NAFLD and NASH, respectively). However, if CDD is kept for long periods of time, oxidative stress, genomic instability, and mutagenesis become frequent phenomena, leading to HCC development. Nevertheless, this process is long and CDD can take up to 1 year to induce HCC in rodents [65]. Feeding with CDD and similar diets (CDD + methionine-free diet, CDD + L-amino acid-defined + high-fat diet [66]) has also been done in combination with other hepatotoxic stimuli (such as CCl_4 or DEN) in order to accelerate tumor formation [67, 68]. Interestingly, very similar tumor gene expression profiles have been observed in high-fat diet + CDD mouse models and better prognosis human HCCs [69], suggesting that this model could mimic less aggressive HCCs. Highly heterogeneous tumor susceptibility is perhaps the greatest limitation of this model.

Peroxisome Proliferators

Ciprofibrate, fenofibrate, methyl clofenapate, and clofibrate are examples of peroxisome proliferators that can promote liver tumorigenesis in rodents. These molecules are ligands of the peroxisome proliferator-activated receptor family (PPAR), which are nuclear receptors that control lipid metabolism in the cell. Their binding to the corresponding receptor stimulates diverse molecular pathways related to oncogenesis, such as cell proliferation (p53, p27, p18, or p21), apoptosis (caspases), metastasis (metalloproteinases and cadherins), and ROS production [70, 71]. The tumors generated after oral administration of these molecules show well-defined HCC histology, with trabecular pattern. However, it seems that the genetic makeup of these tumors is very different to human neoplasms, which implies clear limitations when results are translated into patients. More information about these models can be found elsewhere [29].

Genetically Engineered Mouse Models (GEMMs)

The previously described models are based on the accumulation of genetic alterations through the action of different chemical compounds, which leads to a complex and heterogeneous genetic landscape. Genome editing technologies, however, enable to target specific genes in different model systems, such as mice, thus providing the tools to systematically study the role of different genes and molecular pathways in cancer, including HCC. As a general rule, GEMMs must fulfill several features to be considered good models for HCC research. Essentially GEMMs should recapitulate as many human features as possible. For that, it is optimal to generate models that recapitulate the most frequent genetic alterations occurring in human disease, something that it is not always possible due to the differences between the human and mouse genomes. It is also preferred to restrict these

mutations to a reduced number of hepatocytes, similar to what happens when liver cancer develops in humans. Finally, it would also be ideal to introduce the same mutational load as in human tumors; however, this is technically more challenging since most models only reproduce one or two mutations rather than tens of mutations.

GEMMs can model HCC in many different ways. The most traditional and extended methods involve the in vivo inactivation of TSGs and/or the activation of oncogenes. This can be achieved through diverse genetic engineering strategies. The generation of transgenic mice is the most straightforward option. The transgenic approach is based on the modification of the embryonic genome by transduction of recombinant elements, allowing the expression of oncogenes or dominant-negative TSGs [72]. These inserted genetic elements are in general constitutively and ubiquitously expressed but can also be designed to limit its expression to specific cell types. Conditional expression of transgenes can be achieved through the utilization of tissue-specific promoters, such as the albumin promoter in the context of liver cancer. Transgenic elements can induce non-desirable effects when expressed during embryogenesis or mouse development. To manage the timing of transgene expression and avoid these negative effects, inducible systems based on tetracycline (tet) administration can be used. The tet-inducible system allows the activation or repression of exogenous genes when they are under the tet operon regulating elements (Tet-on and Tet-off systems) [73]. Transgenic models, however, show some limitations. For example, transgene insertion occurs randomly, which could affect the integrity of the landing genes. In addition, the number of copies integrated can lead to an unwanted transgene expression pattern, deeply affecting the final phenotype.

Another interesting strategy is the development of endogenous GEMMs consisting in the targeted loss or gain of function of cancer-related genes. The targeted alteration of selected genes can be achieved by manipulating one or both alleles, in a constitutive or tissue-specific manner, through the employment of Cre-loxP and Cre-loxP-stop recombination mechanisms [74]. Genetic modification can also be induced in adult mouse livers using specific gene transfer procedures, including viral infection or hydrodynamic tail vein delivery. Either way, these models represent invaluable tools for studying human HCC, despite the intrinsic insurmountable differences existing between human and mouse biology. The following is a description of some frequent models recapitulating essential aspects of HCC biology.

Mouse Models of Human Hepatitis

Hepatitis caused by viral infection is the most important risk factor for subsequent HCC development [75]. The host range of hepatitis viruses is mostly limited to humans, which restricts the use of mice as model organisms. As an alternative, GEMMs harboring different components of HBV and HCV have been developed in order to study the effect of these viruses on HCC.

Hepatitis B Virus (HBV) Model

HBV is a DNA virus mainly present in tropical Africa, southeast of Asia, and some areas of China [76]. It is well known that chronically infected HBV patients are more prone to develop HCC due to several factors. First, viral infection can promote hepatocyte dysfunction and the activation of an inflammatory response that causes liver damage, which in turn can stimulate tumor transformation. In addition to this, some of the HBV gene products can be oncogenic per se. But most importantly, HBV DNA gets frequently integrated into the host's genome. HBV integration promotes the rearrangement of adjacent genomic areas, which can affect essential cellular regulatory sequences or key cancer-related genes [77]. Transgenic mice expressing different components of the HBV (surface protein, HBx genes, or even HBV genome) have been established to better understand HBV's underlying biology.

Transgenic mice expressing the surface protein of this virus were the first to be developed and studied. These models have demonstrated that the expression of just this protein is sufficient to induce tumorigenesis in the mouse livers, mimicking some of the human features of the disease and reflecting the different incidence observed in men and women [78]. Expression of mutations in the surface antigens can further increase tumor transformation through mechanisms involving ER stress [79]. Similarly, HBx protein, with effects on almost all basic cellular processes in hepatocytes such as cell division, activation of diverse signaling pathways, mitochondrial function, gene expression, or DNA stabilization, among others, can induce tumoral transformation both in vitro [80] and in vivo [81]. As an example, transgenic mice expressing HBx undergo spontaneous tumor formation 1–1,5 years after birth, presenting human histopathological features (such as trabecular structure, aberrant nuclei, and cirrhotic appearance) and preserving sex incidence discrepancies. Interestingly, no malignant transformation has been observed in models harboring full HBV genome, the precore, or core proteins, highlighting the need of further research to fully understand the role of HBV in HCC, at least in the murine context.

Hepatitis C Virus (HCV) Model

Western countries still present considerable HCV infection rates, being a major risk factor for HCC in these regions. The processes underlying malignant transformation caused by HCV are still unknown, but contrary to HBV, HCV (a positive-strand RNA virus) shows no integration into the human genome. This suggests indirect carcinogenic mechanisms, probably involving the succession of liver injury and healing events after infection together with a persistent inflammatory response, thus resulting in hepatocellular mutagenesis and cancer development over the years [82]. While the accumulation of mutations as a result of liver damage and inflammation is the most accepted explanation for HCV-induced HCC, it is important to note that HCV proteins also seem to have oncogenic effects. In this regard, it has been reported that HCC occurs after 2 years in at least 15% of mice when viral proteins

are expressed [83], but this effect is influenced by the mouse strain. The hepatic expression of core genes, alone [84] or in combination with E1 and E2 structural proteins [85], can also lead to HCC formation although the specific role of the HCV core in liver carcinogenesis is a matter of discussion. This malignant transformation, however, does not occur when envelope (env) or nonstructural genes are expressed [86]. Other models in the context of HCV are the transgenic full-length HCV polyprotein (FL-N) and the HCV structural protein (S-N) mice, which also develop HCC [83] in a steatotic and non-inflammatory background [83].

Mouse Models of Cancer-Related Genes

HCCs arise as a result of the accumulation of mutations in hepatocytes. Unlike other tumor types, the number of genetic alterations and the nature of the pathways regulated by the genes involved are highly diverse, making HCC an extremely heterogeneous disease that is challenging to model. In this respect, whole exome sequencing studies in HCC have shown the presence of at least 40 somatic mutations in coding regions per tumor, affecting both driver and passenger genes [87]. Among the most frequently mutated genes in HCC, we can find some very-well-known players in human cancer such as *TERT* (whose mutation in the promoter is estimated to affect ≈50% of the patients), *MYC* (amplified in ≈20% of the patients), *TP53* (mutated in ≈30% of the patients), or *CTNNB1* (mutated in ≈30% of the patients) [87, 88]. However, there is also a diverse range of mutated genes related to different biological processes such as cell proliferation (*CDKN2A*) or chromatin remodeling (*ARID1A*, *ARID2*, or members of the KMT2 family) that, regardless of their low frequency of mutation in HCC (less than 10%), can still have a significant role in liver tumorigenesis and contribute to the inter-patient heterogeneity [89]. GEMMs can be used for the systematic identification of novel cancer driver genes and the characterization of the pathways involved in HCC malignant transformation and tumor progression. Some of the most interesting and widely used genetic mouse models of HCC are summarized below.

c-MYC

c-MYC (also known as MYC), a member of the MYC family of transcription factors (which includes c-, l-, and n-MYC), is one of the most frequently deregulated genes in human cancers [90]. It is therefore not surprising that high expression levels of *MYC* are a very common feature of human HCC, and *MYC* amplification can potentially be indicative of disease progression [91]. In combination with MAX protein and through the recruitment of histone acetyltransferases, MYC controls the expression of a vast number of genes. When overexpressed (e.g., as a result of gene amplification), MYC leads to the deregulation of closely related pathways (including apoptosis, cell growth, and cell differentiation), thereby resulting in carcinogenesis [92]. In fact, many murine models support this observation. Liver-specific

expression of Myc (driven by albumin promoter) can lead to tumor formation in C57BL/6 J mice after 1,5–2 years [93]. High proliferation rates and p53 dysfunction were observed in this model and contributed to a high mutational load and cancer susceptibility. Similarly, conditional expression of Myc, controlled by the liver activator protein (LAP), also promotes tumor formation in mice and demonstrated oncogene addiction to Myc [94]. Similar approaches have also been tested in more cancer-prone settings, with comparable results. Some notable examples are liver-specific Myc overexpression models in combination with E2F1 or TGF-alpha transgenic expression, which exhibited accelerated HCC formation after 8–9 months [93, 95]. The histological analysis of the transgenic Myc-driven tumors shows characteristics that can be observed in human samples, including trabecular structure, well- and poorly differentiated regions, and morphological and cellular diversity.

β-Catenin (*CTNNB1* Gene)

β-Catenin is a member of the cadherin protein complex and is a key element of the intracellular signaling transduction of the Wnt signaling pathway. Therefore, mutations changing β-catenin functionality promote pro-malignant phenomena such as cell proliferation, apoptosis, cell adhesion imbalance, and altered cell motility [96, 97]. Like MYC, β-catenin is frequently deregulated in an important number of cancer types [90], including HCC [87, 88]. *CTNNB1* gain of function mutations have been described in approximately a quarter of HCC patients [87, 88] and are considered an early event in liver tumorigenesis [98], being traditionally correlated with accelerated tumor progression [99], metastatic transformation [100], and poor prognosis [99]. In this respect, mutations in Wnt/β-catenin pathway can be used for patient stratification, as they correlated with specific gene expression profiles and tumor characteristics [101]. Genetic mouse models have shown that activation of β-catenin is not sufficient to induce HCC, suggesting that additional mutations in the genome are needed for malignant transformation. However, β-catenin activation induces aberrant growth in the liver and hepatomegaly as seen after liver-specific induction of a β-catenin activated form [102]. As an alternative, combination of overexpression of stable forms of β-catenin and other carcinogenic stimuli can induce HCC formation. For example, expression of activated β-catenin together with mutant H-ras overexpression leads to tumor formation in 6 months [103], while activated β-catenin upregulation challenged with DEN administration also induces tumors after 6 months [104].

Cell Cycle Control Genes

Uncontrolled cell growth is a hallmark of cancer [105], and accordingly, alterations in genes regulating cell division are frequent in the vast majority of malignancies, including HCC [87, 88]. To specifically study the role of cell cycle modulators in

HCC, several interesting models have been developed. One major example is the tumor suppressor p53, which plays essential roles in DNA repair, cell growth arrest, and apoptosis induction [106]. Mutations and/or deletions in p53 have been observed in around 30% of all human HCC tumors, a percentage that is higher in patients exposed to aflatoxin B or HCV infection [87, 88, 107]. p53's essential role in liver carcinogenesis is beyond doubt given the high frequency of mutation rates also observed in chemically induced HCC mouse models [108]. However, gene mutations in only p53 are not sufficient to induce liver cancer as it has been seen in different models, but confers aggressiveness features. For example, p53 loss of function in combination with overexpression of the oncogenic polyoma virus middle T antigen (PyMT) confers metastatic capacity to the liver tumors [109]. Similarly, mice expressing just a copy of wild-type p53 undergo tumor formation only after liver damage concomitant with telomerase deregulation [110]. Another example of murine HCC models caused by p53 loss of function includes the conditional p53 and Ink4a/Arf mutant mice injected intrahepatically with polyoma virus middle T antigen (PyMT) [111].

Another key example of liver tumorigenesis induced by deregulation of cell proliferation involves the expression of SV40 virus. The large TAg (T antigen) of SV40 is a well-known oncoprotein which has shown a transforming effect in different contexts by repressing tumor suppressors p53 and retinoblastoma (Rb) and promoting uncontrolled cell proliferation of hepatocytes. Liver-specific induction of SV40-TAg leads to tumor formation in mice after approximately 5 months [112]. Finally, hepatocyte-specific deletion of Rb family members, Rb, p107, and p130, leads to human-like liver tumors after 3–4 months [113] and support the idea of Rb pathway activation as a potential therapeutic option for HCC.

PTEN

The phosphatase and tensin homolog (PTEN) gene is broadly considered as a tumor suppressor [114]. PTEN protein is a phosphatase that exerts multiple functions. On one hand, PTEN inhibits phosphatidylinositol (3,4,5)-trisphosphate – PIP_3 (its major substrate) – which is an activator of the Akt pathway that promotes apoptosis inhibition and cell proliferation [115]. In addition, PTEN loss leads to liver fat accumulation, which is associated with HCC development [116]. On the other hand, PTEN is also involved in chromosomal stability, DNA repair, and cell invasiveness [117, 118]. Despite PTEN mutations not being a common event in HCC cells [87, 88], low levels of PTEN protein are seen in 40–50% of HCC patients and correlate with nonalcoholic fatty liver, increased tumor grade, and advanced tumor stage [119]. In mice, total loss of PTEN function leads to fetal lethality during embryogenesis [120], and while the presence of one functional copy makes them viable, heterozygous mice develop tumors in different organs during their lifetime [121, 122]. Liver-specific PTEN loss, by using albumin-driven Cre recombinase and $Pten^{loxp/loxp}$ mice, enables the induction of hepatomegaly and changes in fat metabolism in the liver, resulting in steatohepatitis [116]. This model not only

reproduces human NAFLD features but also induces the formation of HCC-like tumors in ≈1,5 years, reproducing the gender bias in incidence seen in patients [116].

Telomerase

Telomeres are highly repetitive regions of noncoding DNA located at the end of the chromosomes, being responsible for chromosome stability and preventing loss of genetic information in every cell division [123]. Telomere integrity is maintained by telomerase, TERT, a reverse transcriptase that is activated in very limited number of cell types (mostly in the germline in humans) [124], and TERC, the RNA template for telomere synthesis [123]. As telomeres shorten, the risk of losing important genetic information during cell division increases, inducing senescence or apoptosis in the cell. On the other hand, TERT activation can lead to cell immortalization, which is a key step in malignant transformation. This indicates that TERT can have different roles in tumor initiation and progression.

In the context of liver cancer TERT promoter mutation is the most frequent genetic alteration, affecting around 60% of the patients [87, 88, 98]. In agreement with this, most HCC patients show augmented telomerase activity increased [125, 126]. The role of telomerase dysfunction in liver tumorigenesis is supported by different animal models. One of the most popular is the mTERC-null mice (lacking the telomerase RNA component) [127]. This mouse model, in different oncogenic scenarios, has shown a reduced incidence and growth of HCC in correlation with decreased DNA damage and apoptosis [128, 129], but also associated an augmented number of early malignant lesions in the liver [129], thus confirming the antagonistic effect of telomerase in tumor initiation and progression.

Mouse Models of Inflammation

The importance of inflammation in tumorigenesis is beyond doubt nowadays since it participates in almost every step of cancer development [130]. HCC is the archetypical example of inflammation-driven cancer. Beyond some of the already mentioned viral hepatitis and chemical models (in which there is a significant inflammatory component during tumor formation), there are several GEMMs specifically designed to study the influence of inflammation in HCC. This is the case of those targeting members of the NF-κB pathway. Mice harboring liver-specific deletion of the inhibitor of the nuclear factor Kβ kinase (IKKβ) (in hepatocytes and Kupffer cells), an activator of NF-κB, have shown decrease tumor formation after DEN treatment, demonstrating a promoting role for NF-κB in HCC development [131]. However, when IKKβ loss is restricted to hepatocytes, carcinogenesis is augmented in this same model [131]. Similarly, IKKγ loss in hepatocytes induces tumor formation spontaneously [132], suggesting a dual role for NF-κB in tumorigenesis depending on cellular context. These are just examples

of the complex role of the inflammatory pathways in cancer [133]. Additional GEMMs in the context of inflammation-associated liver cancer are the *Mdr2* knockout mouse [134], the liver-specific TGF-β transgenic mouse [135], and the IL-6 knockout mouse, [44] among others [136].

Non-germline GEMMs

The previously mentioned GEMMs have been extremely helpful in the last years to unveil key concepts of liver cancer biology. However, their generation is very expensive and time-consuming (several months may be necessary from the construction of the targeting vectors to the generation of the first heterozygous mouse) and presents important limitations (some genes are required in embryogenesis). Liver-specific gene delivery methods have been developed in recent years to overcome these disadvantages. Adeno-associated virus (AAV) vectors with liver-tropic capsid are interesting tools, enabling exogenous gene expression in mice [137, 138], and can be used to overexpress oncogenes or other cancer-related genes. Yet, in spite of being a very promising tool, as they are able to induce efficient and time-lasting transduction, its use is limited by several reasons. On one hand, AAV particles usually remain episomal, which leads to gene expression loss in dividing cells. In addition, AAVs present restricted DNA packaging capacity, which restricts the size or the number of genes to deliver. A few years ago, a novel genetic therapy tool called "hydrodynamic gene delivery" was developed as an attractive alternative. This method [139, 140] allows the transduction of genetic elements directly into the murine hepatocytes in vivo by simply injecting naked DNA. In more detail, the procedure involves the injection of exogenous DNA plasmids, resuspended in a very high volume of saline solution, through the tail vein of the mouse. The administration of a massive bolus (corresponding to 1/10 of the mouse body weight) is executed without interruption and rapidly (in no more than 10 seconds), inducing heart congestion and pushing the solution into the liver through retrograde flow. As a consequence, the capillary vessels of the liver undergo high-pressure forces, enabling the permeabilization of the endothelial cells and the transfection of the surrounding hepatocytes with the injected DNA [141]. This method is highly liver-specific reaching transfection levels of up to 40% of the total hepatocytes, barely affecting other organs or cell types [139].

Hydrodynamic gene delivery presents considerable advantages. First, the time and cost of liver-specific GEMM generation is drastically reduced since it only requires the cloning of specific DNA vectors. Related to this, since DNA vector cloning is relatively easy, hydrodynamic gene delivery enables the study of virtually any gene. Similar to conventional GEMMs, the most extended hydrodynamic gene delivery strategies involve the overexpression of oncogenes and/or loss of function of TSGs. For oncogene overexpression, it is important to notice that time-lasting overexpression is required for tumor formation and it can only be achieved through transgene integration into the genome of the hepatocytes. Otherwise, gene overex-

pression would only be transient, lasting no more than a few days before plasmid degradation. The use of DNA plasmids expressing Sleeping Beauty transposon systems is a successful way to ensure plasmid integration [142]. To induce loss of function of TSGs, RNA interference [143] or the recent CRISPR technology [144] can be used. Many HCC models have already been generated using this approach, introducing alterations in p53, β-catenin, MET, or AKT as representative examples [145]. A detailed list can be found elsewhere [145]. Lastly, it is worth noting that, similarly to many GEMMs, most HCC models generated by hydrodynamic gene delivery have been produced in a non-inflammatory background, which is a limiting condition when it comes to closely reproducing human HCC. This suggests the possibility to combine these models with hepatotoxic agent administration in order to better reproduce the conditions that drive human carcinogenesis.

Conclusions and Future Directions

Preclinical models of HCC enable a better understanding of major key processes in human liver carcinogenesis. However, it is also evident that there is no single model that can truly encompass all the genetic and cellular aspects of the human disease. It is therefore essential to deeply understand all currently available models in order to select the most appropriate tools depending on our research interest. Broadly speaking, chemically induced models are suitable to reproduce the liver damage that triggers tumorigenesis in humans. On the other hand, GEMMs allow a more systematic interrogation of the role of specific genes and pathways in liver tumor formation. Transplantation-based models are broadly used tools to study metastasis and test new drug treatments. Finally, in vitro techniques are appropriate to understand the tumor-associated molecular mechanisms and to perform high-throughput experiments (for drug testing or genetic screens). In general, the use of multiple models to address one biological question is optimal as this strategy can overcome the limitations of each individual model. In addition, new challenges in the HCC field, including the study of cancer immunotherapies and precision medicine, warrant the optimization of some of these preclinical models. Our efforts should now focus on improving the existing models to address these new challenges.

References

1. Dhanasekaran R, Limaye A, Cabrera R. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. Hepat Med. 2012;4:19–37. https://doi.org/10.2147/HMER.S16316.
2. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2016;2:16018. https://doi.org/10.1038/nrdp.2016.18.

3. Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, Ye QH, et al. A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(4):187–96. https://doi.org/10.1007/s00432-003-0511-1.
4. Portolani N, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GA, et al. Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. Ann Surg. 2006;243(2):229–35. https://doi.org/10.1097/01.sla.0000197706.21803.a1.
5. Dimitroulis D, Damaskos C, Valsami S, Davakis S, Garmpis N, Spartalis E, et al. From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J Gastroenterol. 2017;23(29):5282–94. https://doi.org/10.3748/wjg.v23.i29.5282.
6. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. J Clin Gastroenterol. 2013;47(Suppl):S2–6. https://doi.org/10.1097/MCG.0b013e3182872f29.
7. Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. J Natl Cancer Inst. 2013;105(7):452–8. https://doi.org/10.1093/jnci/djt007.
8. Collection AATC. Hep G2 [HEPG2] (ATCC® HB-8065™). ATCC. 2016. https://www.atcc.org/products/all/HB-8065.aspx#characteristics. 2018.
9. Lopez-Terrada D, Cheung SW, Finegold MJ, Knowles BB. Hep G2 is a hepatoblastoma-derived cell line. Hum Pathol. 2009;40(10):1512–5. https://doi.org/10.1016/j.humpath.2009.07.003.
10. Bank JC. JCRB0403 – Huh-7. National Institutes of Biomedical Innovation, Health and Nutrition – Japan. 2015. http://cellbank.nibiohn.go.jp/~cellbank/en/search_res_det.cgi?ID=385.2018.
11. Sainz B Jr, TenCate V, Uprichard SL. Three-dimensional Huh7 cell culture system for the study of hepatitis C virus infection. Virol J. 2009;6:103. https://doi.org/10.1186/1743-422X-6-103.
12. Fang C, Yi Z, Liu F, Lan S, Wang J, Lu H, et al. Proteome analysis of human liver carcinoma Huh7 cells harboring hepatitis C virus subgenomic replicon. Proteomics. 2006;6(2):519–27. https://doi.org/10.1002/pmic.200500233.
13. Ali N, Allam H, May R, Sureban SM, Bronze MS, Bader T, et al. Hepatitis C virus-induced cancer stem cell-like signatures in cell culture and murine tumor xenografts. J Virol. 2011;85(23):12292–303. https://doi.org/10.1128/JVI.05920-11.
14. Bressac B, Galvin KM, Liang TJ, Isselbacher KJ, Wands JR, Ozturk M. Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. Proc Natl Acad Sci U S A. 1990;87(5):1973–7.
15. Rebouissou S, Zucman-Rossi J, Moreau R, Qiu Z, Hui L. Note of caution: contaminations of hepatocellular cell lines. J Hepatol. 2017;67(5):896–7. https://doi.org/10.1016/j.jhep.2017.08.002.
16. Ramboer E, Vanhaecke T, Rogiers V, Vinken M. Immortalized human hepatic cell lines for in vitro testing and research purposes. Methods Mol Biol. 2015;1250:53–76. https://doi.org/10.1007/978-1-4939-2074-7_4.
17. Arellanes-Robledo J, Hernández C, Camacho J, Pérez-Carreón JI, editors. In vitro models of HCC, Chapter 42. In: Liver pathophysiology: Academic Press; 2017. https://www.elsevier.com/books/liver-pathophysiology/muriel/978-0-12-804274-8.
18. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nat Cell Biol. 2016;18(3):246–54. https://doi.org/10.1038/ncb3312.
19. Clevers H. Modeling development and disease with organoids. Cell. 2016;165(7):1586–97. https://doi.org/10.1016/j.cell.2016.05.082.
20. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013;499(7459):481–4. https://doi.org/10.1038/nature12271.
21. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015;160(1–2):299–312. https://doi.org/10.1016/j.cell.2014.11.050.

22. Broutier L, Andersson-Rolf A, Hindley CJ, Boj SF, Clevers H, Koo BK, et al. Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. Nat Protoc. 2016;11(9):1724–43. https://doi.org/10.1038/nprot.2016.097.
23. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 2013;494(7436):247–50. https://doi.org/10.1038/nature11826.
24. Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarro LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med. 2017;23(12):1424–35. https://doi.org/10.1038/nm.4438.
25. He L, Tian DA, Li PY, He XX. Mouse models of liver cancer: progress and recommendations. Oncotarget. 2015;6(27):23306–22. https://doi.org/10.18632/oncotarget.4202.
26. Price JE. Xenograft models in immunodeficient animals: I. Nude mice: spontaneous and experimental metastasis models. Methods Mol Med. 2001;58:205–13. https://doi.org/10.1385/1-59259-137-X:205.
27. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc. 2007;2(2):247–50. https://doi.org/10.1038/nprot.2007.25.
28. Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech. 2008;1(2–3):78–82. https://doi.org/10.1242/dmm.000976.
29. Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. Int J Exp Pathol. 2009;90(4):367–86. https://doi.org/10.1111/j.1365-2613.2009.00656.x.
30. Santos NP, Colaco AA, Oliveira PA. Animal models as a tool in hepatocellular carcinoma research: a review. Tumour Biol. 2017;39(3):1010428317695923. https://doi.org/10.1177/1010428317695923.
31. Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. Cancer Metastasis Rev. 1998;17(3):279–84.
32. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017;389(10088):2492–502. https://doi.org/10.1016/S0140-6736(17)31046-2.
33. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018; https://doi.org/10.1016/S1470-2045(18)30351-6.
34. Zimmerman HJ, Lewis JH. Chemical- and toxin-induced hepatotoxicity. Gastroenterol Clin N Am. 1995;24(4):1027–45.
35. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003;349(5):474–85. https://doi.org/10.1056/NEJMra021844.
36. Zhang YJ. Interactions of chemical carcinogens and genetic variation in hepatocellular carcinoma. World J Hepatol. 2010;2(3):94–102. https://doi.org/10.4254/wjh.v2.i3.94.
37. Heath JC. The production of malignant tumours by cobalt in the rat. Br J Cancer. 1956;10(4):668–73.
38. Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther. 1996;71(1–2):57–81.
39. Qi Y, Chen X, Chan CY, Li D, Yuan C, Yu F, et al. Two-dimensional differential gel electrophoresis/analysis of diethylnitrosamine induced rat hepatocellular carcinoma. Int J Cancer. 2008;122(12):2682–8. https://doi.org/10.1002/ijc.23464.
40. Kolaja KL, Klaunig JE. Vitamin E modulation of hepatic focal lesion growth in mice. Toxicol Appl Pharmacol. 1997;143(2):380–7.
41. Tolba R, Kraus T, Liedtke C, Schwarz M, Weiskirchen R. Diethylnitrosamine (DEN)-induced carcinogenic liver injury in mice. Lab Anim. 2015;49(1 Suppl):59–69. https://doi.org/10.1177/0023677215570086.

42. Maronpot RR. Biological basis of differential susceptibility to hepatocarcinogenesis among mouse strains. J Toxicol Pathol. 2009;22(1):11–33. https://doi.org/10.1293/tox.22.11.
43. Nakatani T, Roy G, Fujimoto N, Asahara T, Ito A. Sex hormone dependency of diethylnitrosamine-induced liver tumors in mice and chemoprevention by leuprorelin. Jpn J Cancer Res. 2001;92(3):249–56.
44. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science. 2007;317(5834):121–4. https://doi.org/10.1126/science.1140485.
45. Connor F, Rayner TF, Aitken SJ, Feig C, Lukk M, Santoyo-Lopez J, et al. Mutational landscape of a chemically-induced mouse model of liver cancer. J Hepatol. 2018; https://doi.org/10.1016/j.jhep.2018.06.009.
46. Waxman DJ, Azaroff L. Phenobarbital induction of cytochrome P-450 gene expression. Biochem J. 1992;281(Pt 3):577–92.
47. Watson RE, Goodman JI. Effects of phenobarbital on DNA methylation in GC-rich regions of hepatic DNA from mice that exhibit different levels of susceptibility to liver tumorigenesis. Toxicol Sci. 2002;68(1):51–8.
48. Gourama H, Bullerman LB. Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds: a review. J Food Prot. 1995;58(12):1395–404.
49. Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ Health Perspect. 2010;118(6):818–24. https://doi.org/10.1289/ehp.0901388.
50. Guengerich FP, Johnson WW, Shimada T, Ueng YF, Yamazaki H, Langouet S. Activation and detoxication of aflatoxin B1. Mutat Res. 1998;402(1–2):121–8.
51. Hamid AS, Tesfamariam IG, Zhang Y, Zhang ZG. Aflatoxin B1-induced hepatocellular carcinoma in developing countries: geographical distribution, mechanism of action and prevention. Oncol Lett. 2013;5(4):1087–92. https://doi.org/10.3892/ol.2013.1169.
52. Mace K, Aguilar F, Wang JS, Vautravers P, Gomez-Lechon M, Gonzalez FJ, et al. Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. Carcinogenesis. 1997;18(7):1291–7.
53. McGlynn KA, Hunter K, LeVoyer T, Roush J, Wise P, Michielli RA, et al. Susceptibility to aflatoxin B1-related primary hepatocellular carcinoma in mice and humans. Cancer Res. 2003;63(15):4594–601.
54. Chu YJ, Yang HI, Wu HC, Lee MH, Liu J, Wang LY, et al. Aflatoxin B1 exposure increases the risk of hepatocellular carcinoma associated with hepatitis C virus infection or alcohol consumption. Eur J Cancer. 2018;94:37–46. https://doi.org/10.1016/j.ejca.2018.02.010.
55. Kew MC. Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. Liver Int. 2003;23(6):405–9.
56. Boll M, Weber LW, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch C. 2001;56(7–8):649–59.
57. Bhathal PS, Rose NR, Mackay IR, Whittingham S. Strain differences in mice in carbon tetrachloride-induced liver injury. Br J Exp Pathol. 1983;64(5):524–33.
58. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012;21(4):504–16. https://doi.org/10.1016/j.ccr.2012.02.007.
59. Fitzhugh OG, Nelson AA. Liver tumors in rats fed thiourea or thioacetamide. Science. 1948;108(2814):626–8. https://doi.org/10.1126/science.108.2814.626.
60. Koen YM, Sarma D, Hajovsky H, Galeva NA, Williams TD, Staudinger JL, et al. Protein targets of thioacetamide metabolites in rat hepatocytes. Chem Res Toxicol. 2013;26(4):564–74. https://doi.org/10.1021/tx400001x.
61. Martinez AK, Maroni L, Marzioni M, Ahmed ST, Milad M, Ray D, et al. Mouse models of liver fibrosis mimic human liver fibrosis of different etiologies. Curr Pathobiol Rep. 2014;2(4):143–53. https://doi.org/10.1007/s40139-014-0050-2.
62. Li X, Benjamin IS, Alexander B. Reproducible production of thioacetamide-induced macronodular cirrhosis in the rat with no mortality. J Hepatol. 2002;36(4):488–93.

63. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. Nutr Rev. 2009;67(11):615–23. https://doi.org/10.1111/j.1753-4887.2009.00246.x.
64. Corbin KD, Zeisel SH. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr Opin Gastroenterol. 2012;28(2):159–65. https://doi.org/10.1097/MOG.0b013e32834e7b4b.
65. Chandar N, Lombardi B. Liver cell proliferation and incidence of hepatocellular carcinomas in rats fed consecutively a choline-devoid and a choline-supplemented diet. Carcinogenesis. 1988;9(2):259–63.
66. Ikawa-Yoshida A, Matsuo S, Kato A, Ohmori Y, Higashida A, Kaneko E, et al. Hepatocellular carcinoma in a mouse model fed a choline-deficient, L-amino acid-defined, high-fat diet. Int J Exp Pathol. 2017;98(4):221–33. https://doi.org/10.1111/iep.12240.
67. Kishida N, Matsuda S, Itano O, Shinoda M, Kitago M, Yagi H, et al. Development of a novel mouse model of hepatocellular carcinoma with nonalcoholic steatohepatitis using a high-fat, choline-deficient diet and intraperitoneal injection of diethylnitrosamine. BMC Gastroenterol. 2016;16(1):61. https://doi.org/10.1186/s12876-016-0477-5.
68. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, Martins S, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. J Hepatol. 2018;69(2):385–95. https://doi.org/10.1016/j.jhep.2018.03.011.
69. Hill-Baskin AE, Markiewski MM, Buchner DA, Shao H, DeSantis D, Hsiao G, et al. Diet-induced hepatocellular carcinoma in genetically predisposed mice. Hum Mol Genet. 2009;18(16):2975–88. https://doi.org/10.1093/hmg/ddp236.
70. Borbath I, Horsmans Y. The role of PPARgamma in hepatocellular carcinoma. PPAR Res. 2008;2008:209520. https://doi.org/10.1155/2008/209520.
71. Hsu HT, Chi CW. Emerging role of the peroxisome proliferator-activated receptor-gamma in hepatocellular carcinoma. J Hepatocell Carcinoma. 2014;1:127–35. https://doi.org/10.2147/JHC.S48512.
72. Kersten K, de Visser KE, van Miltenburg MH, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. EMBO Mol Med. 2017;9(2):137–53. https://doi.org/10.15252/emmm.201606857.
73. Lewandoski M. Conditional control of gene expression in the mouse. Nat Rev Genet. 2001;2(10):743–55. https://doi.org/10.1038/35093537.
74. Bouabe H, Okkenhaug K. Gene targeting in mice: a review. Methods Mol Biol. 2013;1064:315–36. https://doi.org/10.1007/978-1-62703-601-6_23.
75. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264–73 e1. https://doi.org/10.1053/j.gastro.2011.12.061.
76. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30(12):2212–9. https://doi.org/10.1016/j.vaccine.2011.12.116.
77. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. J Hepatol. 2016;64(1 Suppl):S84–S101. https://doi.org/10.1016/j.jhep.2016.02.021.
78. Wang Y, Cui F, Lv Y, Li C, Xu X, Deng C, et al. HBsAg and HBx knocked into the p21 locus causes hepatocellular carcinoma in mice. Hepatology. 2004;39(2):318–24. https://doi.org/10.1002/hep.20076.
79. Wang HC, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. Cancer Sci. 2006;97(8):683–8. https://doi.org/10.1111/j.1349-7006.2006.00235.x.
80. Seifer M, Hohne M, Schaefer S, Gerlich WH. In vitro tumorigenicity of hepatitis B virus DNA and HBx protein. J Hepatol. 1991;13(Suppl 4):S61–5.
81. Koike K. Hepatitis B virus HBx gene and hepatocarcinogenesis. Intervirology. 1995;38(3–4):134–42. https://doi.org/10.1159/000150424.
82. Zampino R, Marrone A, Restivo L, Guerrera B, Sellitto A, Rinaldi L, et al. Chronic HCV infection and inflammation: clinical impact on hepatic and extra-hepatic manifestations. World J Hepatol. 2013;5(10):528–40. https://doi.org/10.4254/wjh.v5.i10.528.

83. Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, et al. Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology. 2002;122(2):352–65.
84. Koike K. Molecular basis of hepatitis C virus-associated hepatocarcinogenesis: lessons from animal model studies. Clin Gastroenterol Hepatol. 2005;3(10 Suppl 2):S132–5.
85. Kamegaya Y, Hiasa Y, Zukerberg L, Fowler N, Blackard JT, Lin W, et al. Hepatitis C virus acts as a tumor accelerator by blocking apoptosis in a mouse model of hepatocarcinogenesis. Hepatology. 2005;41(3):660–7. https://doi.org/10.1002/hep.20621.
86. Koike K, Moriya K, Matsuura Y. Animal models for hepatitis C and related liver disease. Hepatol Res. 2010;40(1):69–82. https://doi.org/10.1111/j.1872-034X.2009.00593.x.
87. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet. 2015;47(5):505–11. https://doi.org/10.1038/ng.3252.
88. Cancer Genome Atlas Research Network. Electronic address: wheeler@bcm.edu, Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;169(7):1327–41 e23. https://doi.org/10.1016/j.cell.2017.05.046.
89. Schulze K, Nault JC, Villanueva A. Genetic profiling of hepatocellular carcinoma using next-generation sequencing. J Hepatol. 2016;65(5):1031–42. https://doi.org/10.1016/j.jhep.2016.05.035.
90. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463(7283):899–905. https://doi.org/10.1038/nature08822.
91. Kawate S, Fukusato T, Ohwada S, Watanuki A, Morishita Y. Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic features, proliferative activity and p53 overexpression. Oncology. 1999;57(2):157–63. https://doi.org/10.1159/000012024.
92. Dang CV. MYC on the path to cancer. Cell. 2012;149(1):22–35. https://doi.org/10.1016/j.cell.2012.03.003.
93. Santoni-Rugiu E, Nagy P, Jensen MR, Factor VM, Thorgeirsson SS. Evolution of neoplastic development in the liver of transgenic mice co-expressing c-myc and transforming growth factor-alpha. Am J Pathol. 1996;149(2):407–28.
94. Shachaf CM, Kopelman AM, Arvanitis C, Karlsson A, Beer S, Mandl S, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature. 2004;431(7012):1112–7. https://doi.org/10.1038/nature03043.
95. Calvisi DF, Conner EA, Ladu S, Lemmer ER, Factor VM, Thorgeirsson SS. Activation of the canonical Wnt/beta-catenin pathway confers growth advantages in c-Myc/E2F1 transgenic mouse model of liver cancer. J Hepatol. 2005;42(6):842–9. https://doi.org/10.1016/j.jhep.2005.01.029.
96. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene. 2017;36(11):1461–73. https://doi.org/10.1038/onc.2016.304.
97. Khalaf AM, Fuentes D, Morshid AI, Burke MR, Kaseb AO, Hassan M, et al. Role of Wnt/beta-catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. J Hepatocell Carcinoma. 2018;5:61–73. https://doi.org/10.2147/JHC.S156701.
98. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nat Commun. 2013;4:2218. https://doi.org/10.1038/ncomms3218.
99. Inagawa S, Itabashi M, Adachi S, Kawamoto T, Hori M, Shimazaki J, et al. Expression and prognostic roles of beta-catenin in hepatocellular carcinoma: correlation with tumor progression and postoperative survival. Clin Cancer Res. 2002;8(2):450–6.
100. Lai TY, Su CC, Kuo WW, Yeh YL, Kuo WH, Tsai FJ, et al. β-catenin plays a key role in metastasis of human hepatocellular carcinoma. Oncol Rep. 2011;26(2):415–22. https://doi.org/10.3892/or.2011.1323.

101. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res. 2009;69(18):7385–92. https://doi.org/10.1158/0008-5472.CAN-09-1089.
102. Harada N, Miyoshi H, Murai N, Oshima H, Tamai Y, Oshima M, et al. Lack of tumorigenesis in the mouse liver after adenovirus-mediated expression of a dominant stable mutant of beta-catenin. Cancer Res. 2002;62(7):1971–7.
103. Harada N, Oshima H, Katoh M, Tamai Y, Oshima M, Taketo MM. Hepatocarcinogenesis in mice with beta-catenin and Ha-ras gene mutations. Cancer Res. 2004;64(1):48–54.
104. Nejak-Bowen KN, Thompson MD, Singh S, Bowen WC Jr, Dar MJ, Khillan J, et al. Accelerated liver regeneration and hepatocarcinogenesis in mice overexpressing serine-45 mutant beta-catenin. Hepatology. 2010;51(5):1603–13. https://doi.org/10.1002/hep.23538.
105. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.
106. Kastenhuber ER, Lowe SW. Putting p53 in context. Cell. 2017;170(6):1062–78. https://doi.org/10.1016/j.cell.2017.08.028.
107. Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene. 2007;26(15):2166–76. https://doi.org/10.1038/sj.onc.1210279.
108. Chawanthayatham S, Valentine CC 3rd, Fedeles BI, Fox EJ, Loeb LA, Levine SS, et al. Mutational spectra of aflatoxin B1 in vivo establish biomarkers of exposure for human hepatocellular carcinoma. Proc Natl Acad Sci U S A. 2017;114(15):E3101–E9. https://doi.org/10.1073/pnas.1700759114.
109. Lewis BC, Klimstra DS, Socci ND, Xu S, Koutcher JA, Varmus HE. The absence of p53 promotes metastasis in a novel somatic mouse model for hepatocellular carcinoma. Mol Cell Biol. 2005;25(4):1228–37. https://doi.org/10.1128/MCB.25.4.1228-1237.2005.
110. Farazi PA, Glickman J, Horner J, Depinho RA. Cooperative interactions of p53 mutation, telomere dysfunction, and chronic liver damage in hepatocellular carcinoma progression. Cancer Res. 2006;66(9):4766–73. https://doi.org/10.1158/0008-5472.CAN-05-4608.
111. Chen YW, Klimstra DS, Mongeau ME, Tatem JL, Boyartchuk V, Lewis BC. Loss of p53 and Ink4a/Arf cooperate in a cell autonomous fashion to induce metastasis of hepatocellular carcinoma cells. Cancer Res. 2007;67(16):7589–96. https://doi.org/10.1158/0008-5472.CAN-07-0381.
112. Cullen JM, Sandgren EP, Brinster RL, Maronpot RR. Histologic characterization of hepatic carcinogenesis in transgenic mice expressing SV40 T-antigens. Vet Pathol. 1993;30(2):111–8. https://doi.org/10.1177/030098589303000203.
113. Viatour P, Ehmer U, Saddic LA, Dorrell C, Andersen JB, Lin C, et al. Notch signaling inhibits hepatocellular carcinoma following inactivation of the RB pathway. J Exp Med. 2011;208(10):1963–76. https://doi.org/10.1084/jem.20110198.
114. Hopkins BD, Parsons RE. Molecular pathways: intercellular PTEN and the potential of PTEN restoration therapy. Clin Cancer Res. 2014;20(21):5379–83. https://doi.org/10.1158/1078-0432.CCR-13-2661.
115. Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. Proc Natl Acad Sci U S A. 1999;96(11):6199–204.
116. Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, et al. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest. 2004;113(12):1774–83. https://doi.org/10.1172/JCI20513.
117. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, et al. Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell. 2007;128(1):157–70. https://doi.org/10.1016/j.cell.2006.11.042.

118. Kotelevets L, van Hengel J, Bruyneel E, Mareel M, van Roy F, Chastre E. Implication of the MAGI-1b/PTEN signalosome in stabilization of adherens junctions and suppression of invasiveness. FASEB J. 2005;19(1):115–7. https://doi.org/10.1096/fj.04-1942fje.
119. Hu TH, Huang CC, Lin PR, Chang HW, Ger LP, Lin YW, et al. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. Cancer. 2003;97(8):1929–40. https://doi.org/10.1002/cncr.11266.
120. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. Nat Genet. 1998;19(4):348–55. https://doi.org/10.1038/1235.
121. Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/− mice. Cancer Res. 2000;60(13):3605–11.
122. Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, et al. Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci U S A. 1999;96(4):1563–8.
123. Blackburn EH. Structure and function of telomeres. Nature. 1991;350(6319):569–73. https://doi.org/10.1038/350569a0.
124. Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev. 2002;66(3):407–25, table of contents.
125. Park YM, Choi JY, Byun BH, Cho CH, Kim HS, Kim BS. Telomerase is strongly activated in hepatocellular carcinoma but not in chronic hepatitis and cirrhosis. Exp Mol Med. 1998;30(1):35–40. https://doi.org/10.1038/emm.1998.5.
126. Nagao K, Tomimatsu M, Endo H, Hisatomi H, Hikiji K. Telomerase reverse transcriptase mRNA expression and telomerase activity in hepatocellular carcinoma. J Gastroenterol. 1999;34(1):83–7.
127. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997;91(1):25–34.
128. Lechel A, Holstege H, Begus Y, Schienke A, Kamino K, Lehmann U, et al. Telomerase deletion limits progression of p53-mutant hepatocellular carcinoma with short telomeres in chronic liver disease. Gastroenterology. 2007;132(4):1465–75. https://doi.org/10.1053/j.gastro.2007.01.045.
129. Farazi PA, Glickman J, Jiang S, Yu A, Rudolph KL, DePinho RA. Differential impact of telomere dysfunction on initiation and progression of hepatocellular carcinoma. Cancer Res. 2003;63(16):5021–7.
130. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883–99. https://doi.org/10.1016/j.cell.2010.01.025.
131. Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell. 2005;121(7):977–90. https://doi.org/10.1016/j.cell.2005.04.014.
132. Luedde T, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, et al. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell. 2007;11(2):119–32. https://doi.org/10.1016/j.ccr.2006.12.016.
133. He G, Karin M. NF-kappaB and STAT3 – key players in liver inflammation and cancer. Cell Res. 2011;21(1):159–68. https://doi.org/10.1038/cr.2010.183.
134. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature. 2004;431(7007):461–6. https://doi.org/10.1038/nature02924.
135. Sanderson N, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, et al. Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci U S A. 1995;92(7):2572–6.
136. Newell P, Villanueva A, Friedman SL, Koike K, Llovet JM. Experimental models of hepatocellular carcinoma. J Hepatol. 2008;48(5):858–79. https://doi.org/10.1016/j.jhep.2008.01.008.

137. Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc Natl Acad Sci U S A. 2002;99(18):11854–9. https://doi.org/10.1073/pnas.182412299.
138. Nakai H, Fuess S, Storm TA, Muramatsu S, Nara Y, Kay MA. Unrestricted hepatocyte transduction with adeno-associated virus serotype 8 vectors in mice. J Virol. 2005;79(1):214–24. https://doi.org/10.1128/JVI.79.1.214-224.2005.
139. Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther. 1999;6(7):1258–66. https://doi.org/10.1038/sj.gt.3300947.
140. Zhang G, Budker V, Wolff JA. High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. Hum Gene Ther. 1999;10(10):1735–7. https://doi.org/10.1089/10430349950017734.
141. Kamimura K, Yokoo T, Abe H, Kobayashi Y, Ogawa K, Shinagawa Y, et al. Image-guided hydrodynamic gene delivery: current status and future directions. Pharmaceutics. 2015;7(3):213–23. https://doi.org/10.3390/pharmaceutics7030213.
142. Aronovich EL, McIvor RS, Hackett PB. The Sleeping Beauty transposon system: a non-viral vector for gene therapy. Hum Mol Genet. 2011;20(R1):R14–20. https://doi.org/10.1093/hmg/ddr140.
143. Wuestefeld T, Pesic M, Rudalska R, Dauch D, Longerich T, Kang TW, et al. A Direct in vivo RNAi screen identifies MKK4 as a key regulator of liver regeneration. Cell. 2013;153(2):389–401. https://doi.org/10.1016/j.cell.2013.03.026.
144. Xue W, Chen S, Yin H, Tammela T, Papagiannakopoulos T, Joshi NS, et al. CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature. 2014;514(7522):380–4. https://doi.org/10.1038/nature13589.
145. Chen X, Calvisi DF. Hydrodynamic transfection for generation of novel mouse models for liver cancer research. Am J Pathol. 2014;184(4):912–23. https://doi.org/10.1016/j.ajpath.2013.12.002.

Correction to: Hepatocellular Carcinoma

Yujin Hoshida

Correction to: Y. Hoshida (ed.), Hepatocellular Carcinoma, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8

The book was inadvertently published with incorrect given name and family name in Chap. 4. The author name which is displayed as N.V. Violi is corrected to N. Vietti Violi.

Similarly, the incorrect given name and family name in Chapter 15 is corrected from A.A.R. Suarez to A. A. Roca Suarez.

The updated version of these chapters can be found at
https://doi.org/10.1007/978-3-030-21540-8_4
https://doi.org/10.1007/978-3-030-21540-8_15

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8_17

Index

Y. Hoshida (ed.), *Hepatocellular Carcinoma*, Molecular and Translational Medicine, https://doi.org/10.1007/978-3-030-21540-8