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Liver Cancer

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Meet the editor

Dr. Lasfar is a professor at Rutgers University, New Jersey. He is also editor and board member of several international journals and books. Dr. Lasfar is a scientific adviser to the pharmaceutical industry and a member of several medical and scientific societies. He is also head of the NIH-funded laboratory at Rutgers University, focused on cancer immunotherapy. Dr. Lasfar graduated in Medical and Applied Science from Paris Rene Descartes University in France. He completed his doctoral studies in Immunology at Paris Diderot University and his postdoctoral training in Cancer Immunology at Robert Wood Johnson Medical School, New Jersey.

Contents

Preface

Liver cancer is one of the most common malignancies worldwide and still remains an important public health concern. Hepatocellular carcinoma (HCC) is the most prevalent liver cancer. Although several treatments have been implemented for HCC, their therapeutic efficacy is low and far from overcoming the high recurrence rate of HCC. Therefore, therapy of HCC, particularly the advanced disease, remains a significant unmet clinical need. However, recent advances in immunotherapy using immune check point inhibitors may open a new avenue for HCC treatment.

The majority of patients diagnosed with HCC are elderly people, indicating the vulnerability of the aging population in succumbing to this kind of cancer. Management of HCC in elderly patients is addressed in this book.

HCC originates from hepatocytes, the cells forming the parenchymal tissue of the liver and make up the majority of the liver's mass. Hepatocytes play vital functions in assuming the metabolism of carbohydrates and lipids, protein synthesis, and detoxification from harmful substances. Unfortunately, hepatocytes are subjected to specific viral infections and some products have a high potential to damage liver homeostasis. The factors jeopardizing the normal functions of liver constitute the risk factors for the development of HCC. They interfere in important biological processes, including epigenetic and immune response. All these points are described in detail in this book.

People chronically infected with both hepatitis B and C present a higher risk for developing HCC. There are ~300–350 million carriers of hepatitis B virus worldwide. For this reason, in this book, we have shown a particular interest in hepatitis B virus.

Currently, many options are available for the treatment of HCC. Potentially curative treatments like surgical resection or liver transplantation might be possible for less advanced HCC. Those options are discussed in this book. Unfortunately, advanced HCC remains an urgent unmet clinical need. However, novel clinical trials with immune check inhibitors indicate that new hopes are around the corner for the treatment of advanced HCC. This point is addressed in this book.

Important efforts and collaborations with leading experts in the field were crucial for achieving this high-quality book. We thank all the contributors for sharing their expertise, expressing their views, and also bringing new hopes for this devastating disease. The readers will appreciate the excellent and reliable information this book offers.

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Introduction

Chapter 1

Introductory Chapter: Liver Cancer, Risk Factors and Current Therapies

Ahmed Lasfar

Additional information is available at the end of the chapter

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1. Introduction

Liver cancer is one of the major cancers in the world [1]. Hundreds of thousand people are diagnosed each year with liver cancer. Unfortunately, liver cancer is the second most common cause of deaths associated with cancer complications, accounting for more than 70%. Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer [2]. More than two-third of patients newly diagnosed with HCC are aged >65 years, and this number is expected to increase as the world population ages [3]. Furthermore, there is heterogeneity in the aging process, which further contributes to the complexity of treatment decisions [4, 5].

HCC originates from normal hepatocytes. Hepatocytes are the cells forming the parenchymal tissue of the liver and make up the majority of liver's mass. Hepatocytes play a crucial role in liver functions [6]. They are involved in many biological processes including the metabolism of carbohydrates and lipids, protein synthesis, and notably body detoxification from harmful substances. Important proteins such as serum albumin, prothrombin, transferrin, fibrinogen, and complement are generated by hepatocytes. In addition to their main role in glycogenesis, hepatocytes make fatty acids from carbohydrates leading to triglyceride synthesis. Hepatocytes are highly involved in lipid metabolism and cholesterol synthesis. The detoxifying activity of hepatocytes includes drug metabolism, modification of endogenous compounds such as steroids and ammonia. However, hepatocytes might be overwhelmed with harmful agents and targeted with many hepatic viruses, leading to liver damage and ultimately to HCC [7]. The hepatocytes are commonly used for research in both academia and pharmaceutical industry in order to investigate the mechanisms of carcinogenesis, viral infections, and drug metabolism. Currently, highly innovative research in epigenetics and immunology is taken place in order to explore further liver diseases and develop novel therapies for HCC.

2. Epigenetic of HCC

Epigenetic modifications are crucial in HCC. They arise in the context of known risk factors leading to chronic liver disease and concern mostly chemical alterations of DNA and histones. DNA methylation is the commonly investigated, showing its relevance in the mechanisms of gene silencing. Currently, genome-wide methylation analysis indicates important changes in the methylation status of oncogenes, signaling molecules, and suppressor genes [8]. Therefore, targeting the epigenome could lead to novel therapies of HCC.

3. Immunogenicity of HCC

Tumor immunogenicity of HCC has been first demonstrated by using autologous tumor lysate and dendritic cells for the prevention of recurrence in HCC patients. Subsequently, several tumor-associated antigens (SART2, CypB, SART3, AFP p53, MRP3, and hTERT) have been identified and characterized in HCC, suggesting the development of highly effective immunotherapy [9, 10].

The modulation of immune costimulatory molecules has been also shown to play critical role in the pathogenicity of the liver. The costimulatory ligand member B7 is a crucial immune checkpoint in HCC [11]. B7-1, B7-2, B7-DC, and B7-H1 are expressed on professional antigen-presenting cells and regulate T cell activation after the binding with CD28, CTLA-4, or PD-1. B7-H3 is expressed in human HCC cells and is associated with tumor aggressiveness and post-operative recurrence [12]. Apparently, B7-H3 promotes aggression and invasion of HCC by targeting epithelial-to-mesenchymal transition via JAK2/STAT3/Slug signaling pathway [13].

4. Common risk factors for HCC

The major common risk factors for HCC are hepatic virus infection with HBV and HCV. Fatty liver disease, related or unrelated to alcohol abuse which frequently lead to liver cirrhosis, is the other major condition, increasing the risk for developing HCC (**Figure 1**).

4.1. Hepatitis B virus (HBV) infection

HBV is one of the most common etiologic factors leading to HCC worldwide. The risk of developing HCC is more than 15-fold in patients with HBV chronic infection [2, 14]. In most developed countries, around of 10% of HCC is associated with HBV infection which occurs through either parental contact with infected blood or sexual transmission. In contrast, other geographic regions in the world where HBV is endemic such as sub-Saharan Africa and Asia, HBV transmission occurs mainly via perinatal exposure [15, 16].

HBV patients are highly prone to secondary infection with hepatitis D virus (HDV). The HDV is dependent on HBV genome products to form its own. Infection with HDV is more frequent

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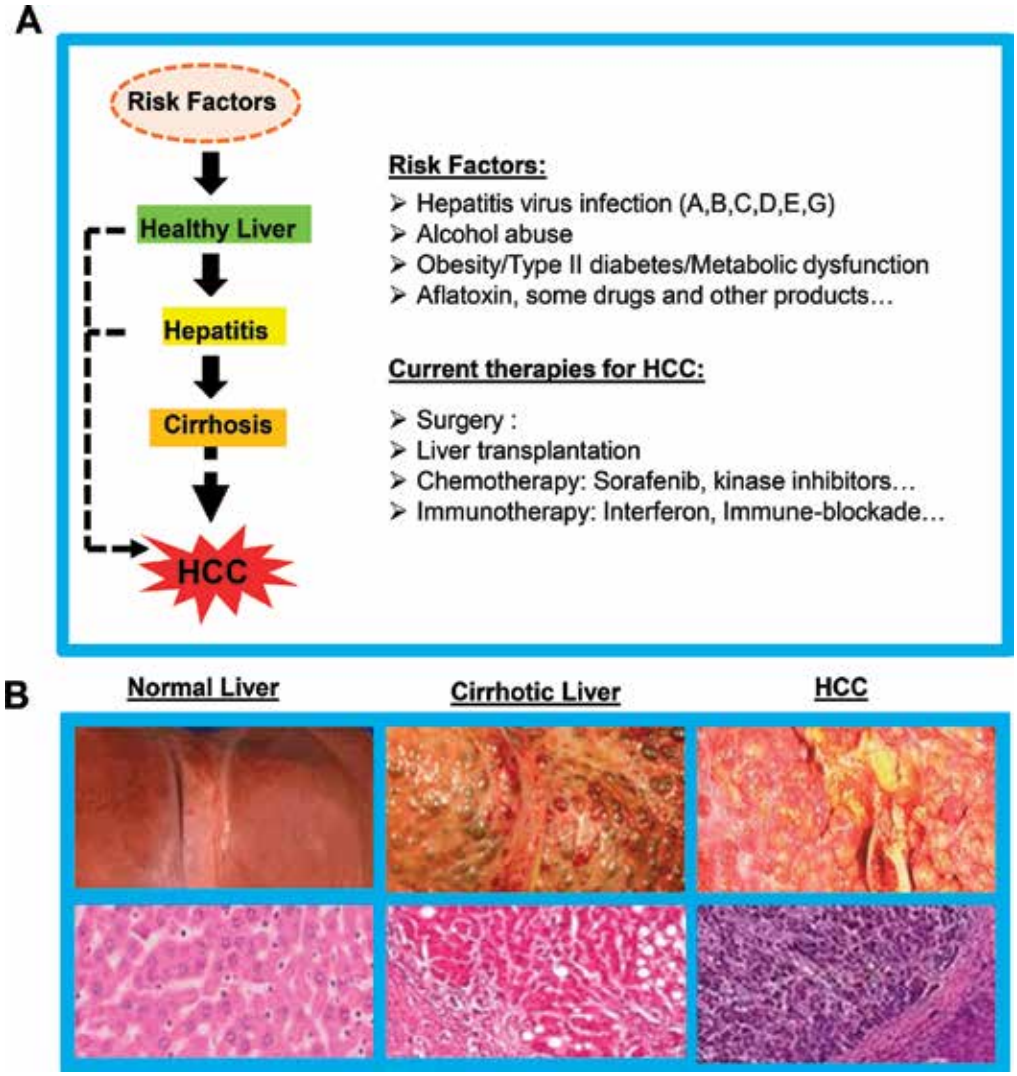

Figure 1. Risk factors and current therapies of liver cancer. Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer. A. Hepatocytes, the main liver cells are subjected to harmful conditions (hepatitis virus infections) causing hepatitis and frequently leading to cirrhosis and ultimately to HCC. Many treatment options are currently available for HCC. B. Gross anatomy and histology of healthy liver and liver diseases related to cirrhosis and HCC.

in sub-Saharan Africa, Mediterranean regions, and South America [17]. At least eight HDV genotypes, geographically distributed in different regions of the globe, have been reported. An estimated 20 millions of people are infected with one of HDV genotype. In combination with HBV infection, HDV precipitates liver failure and HCC [18]. The best treatment for HDV infection is the eradication of HBV through HBV vaccine.

Currently, 10 HBV genotypes have been described. Apparently, HBV-infected patients with C and D genotypes develop more frequently liver cirrhosis and HCC than HBV patients,

infected with the other genotype strains. Furthermore, those patients respond poorly to current therapies based on interferon or other antiviral agents [15, 16].

4.2. Hepatitis C virus (HCV) infection

HCV infection is also one of the frequent risk factors in developing HCC in the word. The risk of HCC is very high in patients chronically infected with HCV [19]. Coinfection with HIV or HBV increased this risk further. The high majority of coinfected patients with either HIV or HBV precipitate chronic hepatitis, leading to HCC. Suppression of HCV load by IFN therapy, apparently, participates in reducing the onset of HCC [20]. However, concerns regarding the impact of HCV direct-acting agents (DAAs) on the incidence of HCC continue to be raised in clinic [21]. The potential increased risk of HCC in HCV patients under DAAs therapy has been reported [22]. Therefore, interferon therapy should not be discontinued at least for HCV patients with high risk of HCC.

4.3. Alcohol abuse

Evidence shows that long-term alcohol use is responsible for alcoholic liver disease (ALD) and a high risk of developing HCC [23]. ALD is well characterized; however, little progress has been made for its treatment. It is well established that alcohol is highly toxic to hepatocytes. By causing continuous cell necrosis, it induces perpetual regeneration of hepatocytes and paves the way to carcinogenesis [24]. In addition, alcohol causes liver damage by promoting inflammation that precipitates cirrhosis and leads to HCC [23]. The effect of alcohol on liver disease is boosted in people with viral hepatitis [25].

4.4. Nonalcoholic steatohepatitis (NASH)

NASH is a condition of fatty liver disease in which liver has abnormal fat accumulation and increased inflammation. Although the exact etiology of NASH remains unknown, the risk factors include obesity, type II diabetes, and related metabolic dysfunctions. NASH patients with no cirrhosis have no increased risk of HCC, indicating that induction of liver cirrhosis is a leading cause of HCC. However, the outcome of NASH is much similar as other chronic hepatitis such as HCV infection [26]. Although the risk of developing HCC might be lower in NASH patients than HCV patients, the severity of HCC and patient survival in both cases remain similar.

5. Liver cirrhosis and other risk factors for HCC

The majority of HCCs arise from liver cirrhosis, a condition in which liver tissue is replaced by scar tissue [27]. The scar tissue jeopardizes the blood flow through the liver and retains it from functioning correctly. Cirrhosis results mainly from different chronic hepatitis mainly due to viral infections and fatty liver disease related or unrelated to alcohol abuse. Currently, besides HBV, HCV, and HDV, three hepatitis viruses are identified and have been demonstrated to

induce hepatitis: hepatitis A virus (HAV), hepatitis E virus (HEV), and hepatitis G virus (HGV). However, HBV and HCV are the most common inducers of hepatitis-related virus infections. People chronically infected with both hepatitis B and C present higher risk for developing HCC.

Besides hepatitis virus infection and fatty liver disease related or unrelated to alcohol consumption, aflatoxin has been shown to increase the risk of developing HCC [28]. Aflatoxin is a family of fungus toxins that could be present at high levels in frequently consumed food such as nuts, grains, and spices that are not adequately selected or properly stored. Aflatoxin enters the food supply and can be found in animal and human-processed foods. Animals can pass aflatoxin derivative products into milk, eggs, and meat. Overweight and obesity constitute other independent risk factors for HCC. Therefore, in order to efficiently prevent hepatitis and HCC, raising awareness through general public education should be highly supported [29].

6. Treatment options for HCC

Currently, many options are available for the treatment of HCC [30]. Potentially curative treatments like surgical resection or liver transplantation might be possible for less advanced HCC. Minimally invasive surgical technologies continue to improve increasing its safety and applicability for oncologic liver surgery. Different surgical procedures, including advanced surgical technologies, are currently performed.

Unfortunately, tumor recurrence and metastasis frequently occur after resection and limit the overall survival. In patients with unresectable HCC and preserved liver function, transarterial chemoembolization (TACE) can prolong survival. However, TACE is rarely curative. More than half of patients with HCC continue to die secondary to liver failure from progressing cirrhosis. Current chemotherapy, interferon treatment, or alternative medicine only partially benefits patients with advanced disease. Therefore, novel treatments for liver cancer, particularly advanced HCC, are in urgent need [31].

Since the introduction of sorafenib, a multikinase inhibitor that showed some benefits to HCC patients, other targeted and immune therapies emerged for the treatment of HCC. Currently, promising therapies for HCC are underway, including targeted therapy, immune checkpoint inhibitors, oncolytic viruses (OVs), and chimeric antigen receptor-redirected T cells (CAR-T cells). Combination strategies are also under investigation to promote further the treatment of advanced HCC [32].

7. Emergence of immune checkpoint inhibitors

HCC patients with advanced disease, not eligible for currently curative procedures, particularly surgery or local interventions, were selected to test the efficacy of immune checkpoint inhibitors in clinical trials [11]. CTLA-4 blockade with tremelimumab showed a high promise

for controlling the tumor in patients with advanced HCC and HCV infections. This new therapeutic strategy opened the way for testing other immune checkpoint inhibitors, controlling other pathways such as PD-L1/PD-1. Furthermore evidences showing high expression of PD-L1/PD-1 in HCC patients support the use of PD-L1/PD-1 inhibitors. Indeed the result of PD-1 blockade with anti-PD-1 antibody (nivolumab) in a large phase II trial, regrouping HCC patients resistant to sorafenib is very promising [33, 34].

Although immunotherapy for HCC seems promising, important concerns regarding the selection of patients that could mostly benefit from this therapy are now under intensive investigation. In this regard, the mechanisms of resistance to immune checkpoint inhibitors and the identification of markers, predicting the response to immunotherapy need to be considered in selecting patients for treatment [35, 36].

In conclusion, promising results with immune blockade inhibitors have been currently published in HCC clinical trials, using anti-CTLA-4 agent tremelimumab and anti-PD-1 agent nivolumab. We believe that in the near future, immune-based therapies and combination with chemotherapeutic agents will bring a paradigm shift for treatment of advanced HCC.

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Section 2

Liver Pathogenesis and Cancer Development

Chapter 2

Pathogenesis of Hepatitis B Virus Associated Chronic Liver Disease

Mark A. Feitelson

Additional information is available at the end of the chapter

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Abstract

Hepatitis B virus (HBV) infection is associated with chronic liver diseases (CLD), which progress from hepatitis to fibrosis, cirrhosis, and finally hepatocellular carcinoma (HCC) over 30–50 years. The pathogenesis of CLD is immune mediated, which is characterized by persistent immune responses against virus infected hepatocytes. During bouts of CLD, the virus gene encoding the hepatitis B x antigen (HBx) is increasingly found integrated at multiple sites within the human genome. Many of these integrated templates express HBx, which is a *trans*-regulatory protein that supports virus gene expression and replication on one hand, but also alters patterns of gene expression in the infected cell. HBx alters gene expression by constitutively activating signal transduction pathways in the cytoplasm and promoting epigenetic mediated changes in the expression of cellular genes. In doing so, HBx contributes to the persistence of virus infected cells and to the pathogenesis of CLD by triggering multiple hallmarks which are characteristic of cancer.

Keywords: hepatitis B virus, chronic liver disease, hepatocellular carcinoma, hepatitis B x, immune mediated liver disease, epigenetics, hallmarks of cancer

1. Introduction

Hepatitis B virus (HBV) is a blood-borne virus that infects the liver. Until the discovery of the virus in the 1960s [1], it was transmitted sexually and by transfusion of contaminated blood and blood fractions. Today, the virus has been virtually eliminated from the blood supply by a simple blood test while infection has been prevented by a highly efficacious vaccine [2, 3]. Prior to establishment of vaccination programs in various countries, infants born to infected mothers replicating virus often acquired the virus at birth by exposure to contaminated maternal blood.

More than 90% of these children became HBV carriers, characterized by the persistence of virus or virus antigens in their blood for years to decades. These children were a high risk for the development of chronic liver disease (CLD), which progressed from hepatitis, to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [4]. Fortunately, newborns in many countries receive the HBV vaccine at birth, which helps to prevent mother-to-infant transmission as well as protect from exposure later in life. Among unvaccinated adults engaging in unprotected sex, roughly 5–10% become carriers, and these individuals are also at high risk for the development of CLD and HCC. Although estimates vary, there are ~300–350 million carriers of HBV worldwide [5]. HCC is the sixth most prevalent cancer worldwide, with about 600,000 newly diagnosed cases annually, and the second leading cause of cancer deaths [6]. Interferon, and in more recent years, powerful nucleoside analogs, have successfully treated patients with chronic hepatitis B, but presently there is no cure [7, 8]. HCC is curable by surgical resection, but this is often accompanied by relapse. Dozens of drugs, alone or in combination, have been evaluated in clinical trials for patients with advanced HCC, but only the multi-kinase inhibitors, Sorafenib and regorafenib, and the immune checkpoint inhibitor nivolumab, have been useful in modestly extending the lifespan of such patients [9, 10]. Given that the carrier state and CLD are the major risk factors for HCC [11], there is strong rationale to better understand the host-virus relationship that contributes to the pathogenesis of chronic infection.

2. Variations in pathogenesis

A hallmark in the pathogenesis of HBV infection is its' variability. Among acutely infected adults, up to 65% develop a subclinical infection characterized only by the appearance of one or more viral antibodies in the blood, while another 25% develop acute resolving infection, which may or may not include a bout of hepatitis. The remaining 10% of patients develop chronic infection (i.e., the persistence of virus and virus antigens in the blood for more than 6 months). In chimpanzees [12] and woodchucks [13], acute infections are characterized by the nearly complete clearance of virus from the blood and liver followed by seroconversion from surface antigen to corresponding antibody. In this case, virus is mostly cleared by non-cytolytic cytokines (e.g., interferon gamma [IFNɣ] and tumor necrosis factor alpha [TNFα]) prior to the appearance of T and other inflammatory cells in the liver, suggesting that most virus clearance occurs prior to the development of acute hepatitis. Further work showed that $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, Fas, various IFNs and corresponding receptors, and the TNF receptor 1 participate in virus clearance, suggesting redundant pathways inhibit HBV replication in the liver [14]. The subsequent contribution of a T cell response appears to clear virus infected cells by cytolytic mechanisms involving Fas and granzymes. In this context, $CD4^+$ T cells are required to prime $CD8^+$ T cells to facilitate virus elimination in acute infection [14]. When this happens in acute, resolving infection, the T cell response to HBV is vigorous, polyclonal and multi-specific, while among those who go on to develop chronic infection, adaptive immunity is relatively weak and narrowly focused, suggesting that clearance of HBV is T cell dependent. When T cell responses are not adequate, CLD may develop and progress to cirrhosis and HCC. However, CLD may spontaneously resolve at any of these stages. While the origin of this variability is not completely characterized, it is clear that the ability of the host to mount adaptive immune responses is a key element to limiting virus spread.

3. Contributions of hepatitis B surface, core and e antigens to the pathogenesis of chronic infection

3.1. Hepatitis B surface antigen (HBsAg)

HBV is a small virus consisting of only four open reading frames (ORF) [15]. One ORF encodes a family of envelope polypeptides (**Figure 1**). The major envelope polypeptide, HBsAg, triggers neutralizing antibody which is central to virus clearance after acute exposure and is the major component of the HBV vaccine [2]. HBsAg polypeptides are transmembrane proteins and glycoproteins that are on the envelope of virus particles, and are also secreted as small, spherical and variably long filamentous subviral particles that lack the virus nucleocapsid and HBV DNA. It is thought that these subviral particles, which are produced at concentrations several logs above that of infectious virus particles, absorb neutralizing antibody and trigger immunological tolerance, both of which promote virus persistence in the blood. Moreover, in patients with CLD, there does not seem to be any correlation between intrahepatic HBsAg expression patterns and inflammatory infiltrates [16, 17], nor have HBsAg specific T cell clones been isolated from such patients [18]. In addition, T cell sensitization to HBsAg in acute and chronic HBV infection is usually undetectable [19],

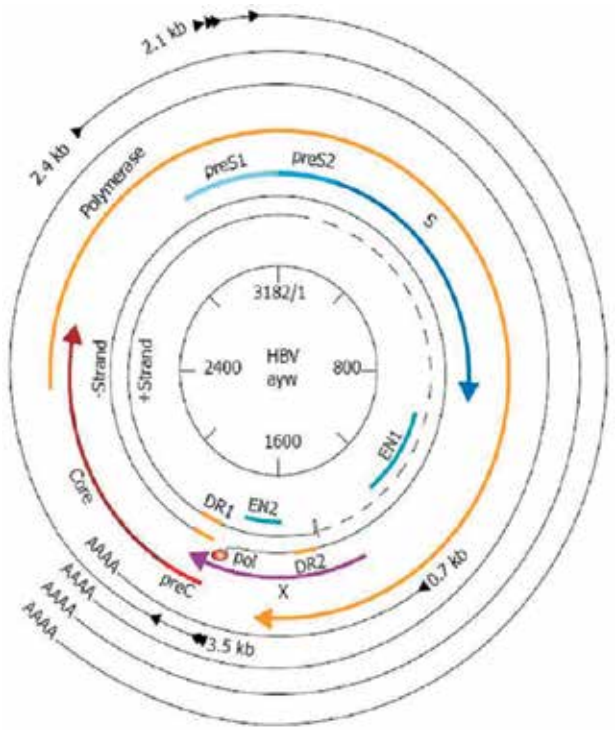

Figure 1. Genetic organization of HBV showing the ORFs (in color). The positions of enhancer 1 (EN1) and 2 (EN2) are also shown. The direct repeat 1 (DR1) and 2 (DR2) sequences at the ends of the long and short DNA strands are also indicted. The pregenomic RNA (3.5 kb) is greater than genome length, while the 2.1 and 2.4 kb subgenomic mRNAs encode surface antigen polypeptides, and the 0.7 kb mRNA encodes the X protein. Reproduced from [20] with permission.

so while HBsAg clearance occurs in acute, resolving infections, it is not clear that it is an immunological target in established infections.

3.2. Hepatitis B core antigen (HBcAg)

The second ORF, or core gene, encodes the hepatitis B core antigen (HBcAg) or nucleocapsid protein that polymerizes as an icosahedron around the virus replication complex, the latter of which consists of the virus nucleic acid and HBV encoded polymerase [20]. The fact that the pregenomic RNA and the reverse transcribed viral DNA product are sequestered within a nucleocapsid means that they are not readily detected by pattern recognition receptors, (e.g., toll-like receptors, retinoic acid inducible gene 1 [RIG-1], and mitochondrial anti-viral signaling [MAVS]) that trigger innate immunity [21]. Moreover, innate immune responses do not develop in the liver of acutely infected chimpanzees [22], suggesting that HBV replication and spread may be conducted in "stealth" mode with virus nucleocapsids upon infection and again during virus replication. If so, then this may explain why up to 70% of acutely infected adults who become carriers do not develop CLD. However, carriers who develop CLD also have intrahepatic core antigen, suggesting that HBcAg may be an important immunological target in CLD [23]. Alternatively, patients with acute, resolving hepatitis show a vigorous peripheral blood mononuclear cell response to HBcAg that is temporally associated with the clearance of HBsAg, while in patients with chronic infection, T cell responsiveness to HBcAg is relatively weak, providing an opportunity for HBV to spread in the liver and establish a chronic infection [19].

3.3. Hepatitis B e antigen (HBeAg)

A proteolytic fragment of HBcAg, known as HBeAg, is secreted into the circulation and serves as a surrogate marker of virus replication. Seroconversion from HBeAg to anti-HBe is usually accompanied by a significant decrease in virus replication in both the liver and blood and resolution of CLD [24]. The detection of HBcAg specific cytotoxic T lymphocytes (CTL) is associated with the clearance of virus replication, often a transient exacerbation of CLD, and seroconversion to anti-HBe during the natural history of infection [24], suggesting that HBcAg is an important virus target in CLD. HBcAg specific T cells have been detected in the peripheral blood and liver [18, 25] of patients with CLD, suggesting that HBcAg is an immunological target in chronic hepatitis B. Interestingly, HBeAg in serum may attenuate immune responses against virus infected liver, because some patients who develop mutations in HBV that no longer express HBeAg, continue to support high levels of virus replication and ongoing, CLD [26, 27]. In fact, HBeAg appears to be a T cell tolerogen that down-regulates immune responses against HBcAg [28]. HBeAg may also stimulate the appearance of regulatory dendritic cells, which would also suppress virus specific immunity and promote virus persistence [29] by up-regulating the expression of suppressor of cytokine signaling 2 (SOCS2), which in turn represses IFN signaling, thereby blunting innate anti-viral responses and promoting virus persistence [30]. Thus, HBeAg polypeptides, like subviral HBsAg particles, promote chronicity by acting as tolerogens.

3.4. Hepatitis B polymerase

The HBV encoded polymerase, encoded by a third ORF, has DNA dependent and RNA dependent DNA polymerase (DNAp) activities, and RNase H activity. Upon infection, the partially

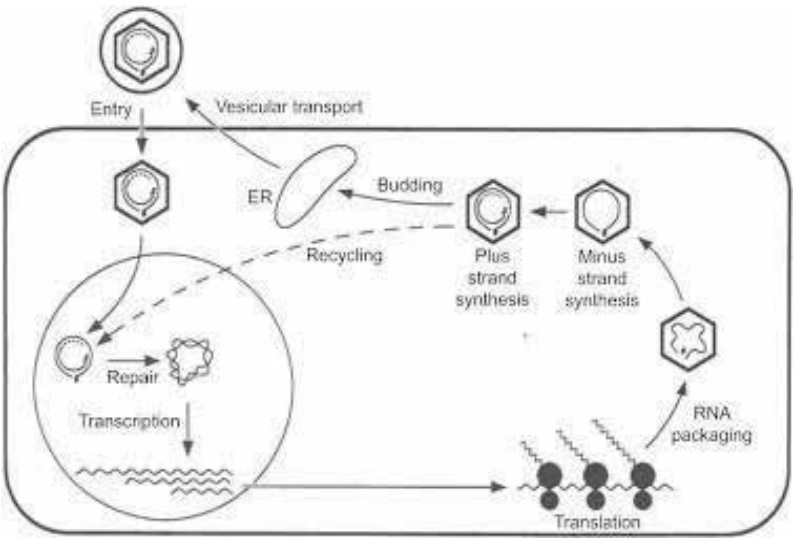

Figure 2. General scheme of HBV replication. See the text for additional details. Reproduced from [20] with permission.

double stranded viral DNA is made fully double stranded by the endogenous DNAp activity [20] (**Figure 2**). The HBV genome then appears as a supercoiled mini-chromosome in the nuclei of infected cells, and this acts as a template for the transcription of subgenomic RNAs and a greater than genome length pre-genomic RNA. The latter then migrates into the cytoplasm, where it is packaged with the virus polymerase into nascent ("immature") core (or nucleocapsid) particles, where the pregenomic RNA is reverse transcribed into minus strand DNA, with the latter then being used as a template for partial plus strand synthesis just prior to the budding and secretion of progeny virus (**Figure 2**). Some immature core particles are recycled into the nucleus to replenish the pool of covalently closed circular (ccc) HBV DNA. Although the HBV polymerase triggers antibody responses [31], there is no evidence that immune responses against the polymerase directly impact pathogenesis or virus persistence. However, HBV polymerase inhibits RIG-1 and nuclear factor kappa B (NF-κB) induction of IFNβ, suggesting that the polymerase could block innate signaling [32, 33], thereby contributing to virus persistence.

4. Relationship between persistent virus replication, integration of HBV DNA, and the risk for the development of HCC

There is evidence to suggest that persistent, high levels of HBV replication correlate with the progression of CLD to HCC [34]. However, independent work showed an elevated risk for HCC among patients with CLD but low virus titers [35, 36]. Other observations have shown no correlation between HBV DNA levels in serum (>10^5 copies/ml) and histological grade or stage of liver disease in carriers [37, 38]. In addition, it is controversial as to whether long term nucleoside analog therapy resulted in a decreased risk for the development of HCC [6, 35]. Given that HBV is not directly cytopathic [39], that carriers with high levels of HBV DNA in serum are often asymptomatic, and that the pathogenesis of CLD is immune mediated [17,

40], a correlation between virus replication and CLD may contribute to, but not by itself, determine disease progression. Moreover, most carriers with CLD who develop cirrhosis and HCC have long since seroconverted from HBeAg (reflecting high levels of virus replication) to anti-HBe (reflecting low or undetectable virus replication), indicating that disease progression may occur at low virus titers [36]. Among patients with sustained high levels of HBV replication and successive bouts of CLD, there is a wave of liver regeneration following each episode of hepatitis to restore full liver function. At these times, fragments of HBV DNA, mostly encoding the HBx ORF (and sometimes the HBx plus preS/S ORFs), become integrated at multiple sites within host DNA [41, 42] (**Figure 3**). Over time, these integration events result in increased intrahepatic expression levels of HBx that alter patterns of host (and support virus) gene expression (**Figure 3**). HBV integrates early after infection, not only in permissive liver cell lines, but also in non-replicating primary human hepatocytes [43]. Many fragments of integrated HBV DNA encode HBx that is capable of *trans*-activation [44]. Although the relatively low levels of HBx made from the virus mini-chromosome support virus gene expression and replication, it is hypothesized that as intrahepatic levels of HBx increase [45] (**Figure 3**), it epigenetically alter the expression patterns of selected host genes [46] that contribute to both virus persistence and to malignant transformation. Thus, the changing intrahepatic levels of HBx promote virus persistence and ultimately, contribute to malignant transformation [47].

4.1. Covalently closed circular HBV (ccc) DNA

Given that the current treatment of chronic hepatitis B with nucleoside analogs is not curative, there has been a major effort to eliminate ccc DNA [47], especially since ccc DNA is the template for all virus transcripts. Since nucleoside analogs do not eliminate integrated HBV templates or the HBV mini-chromosome, continued virus gene expression from these templates will drive pathogenesis toward HCC. Formation of ccc DNA is a complex process that involves a variety of host proteins, including several DNA polymerases [48] that could potentially be therapeutic targets, although this approach may be accompanied by toxicity. As outlined below, HBx regulates the formation, function and intracellular copy number of ccc DNA by several epigenetic mechanisms that involve altered expression of histone methyltransferases and histone deacetylases, by promoting degradation of the anti-viral restriction factor Smc5/6, and by increasing expression of DNA methyltransferases [48]. Anti-viral immune responses in which selected cytokines mediate non-cytolytic degradation of ccc DNA have also been documented *in vitro* [48, 49]. Among these, IFN alpha up-regulated expression of APOBEC3

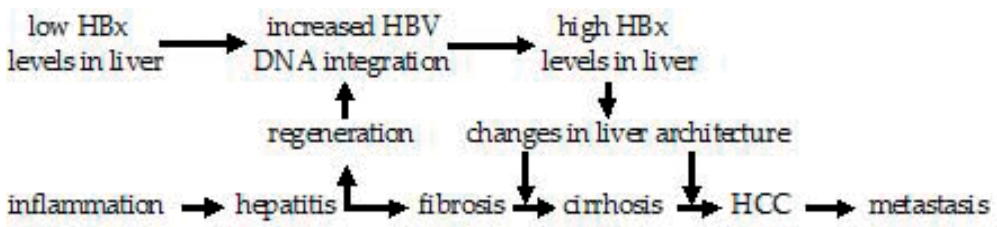

Figure 3. Natural history of chronic hepatitis B featuring the progressive lesions that develop in CLD compared to increased number of integration events, many of which produce functional HBx (modified from [53] with permission).

nuclear deaminase resulted in a modest reduction in ccc DNA copy number via deamination [50]. Gene editing approaches, such as CRISPR/Cas9 have also been demonstrated to work *in vitro* and *in vivo* [51], but off-target effects, ability to access and act on all susceptible cells, and recognition of all HBV genotypes, remain to be addressed. In addition, the recent finding of ccc host DNA in both normal and tumor cells, as a mechanism whereby host cells regulate gene expression [52], implies that targeting ccc DNA may also have toxic effects on the treated cells whether or not they are virally infected. Thus, it is not clear whether this approach in a liver which is already damaged will exacerbate that damage and/or have an anti-tumor effect.

5. Contribution of HBx to pathogenesis of CLD by regulation of HBV replication

HBx, the *trans*-activation protein of HBV, *trans*-activates virus gene expression and replication *in vitro* [54, 55]. The contribution of this regulatory protein to virus persistence in the carrier state was shown in woodchucks experimentally infected with the HBV-like virus, woodchuck hepatitis virus (WHV). Wild type WHV readily establishes a chronic infection, characterized by persistent virus replication and CLD that progresses to HCC [56]. However, experimental infection with a mutant of WHV that does not encode woodchuck hepatitis x (WHx) antigen yielded no carrier state and no CLD [57, 58], suggesting that *trans*-activation of virus gene expression and replication is central to the establishment of the carrier state. Among infected woodchucks, there was co-staining between WHV core antigen (where virus replication takes place) and WHx [59, 60], while in human infection, HBx often co-existed with HBe in serum [61] and replication complexes (i.e., with HBcAg) in the liver [62]. Thus, HBx expression is associated with virus replication.

5.1. Mechanisms regulating HBV replication

At the molecular level, HBx regulates HBV replication by binding to various cellular proteins. For example, HBx binds to jumonji C-domain-containing 5 (JMJD5), a arginyl-hydroxylase, which promotes the expression of transcription factors (e.g., such as hepatocyte nuclear factors 3 gamma and 4 alpha [HNF3G and HNF4A] and CCAAT/enhancer-binding protein alpha) that facilitate hepatocyte differentiation [63]. Given that HBV replicates in differentiated hepatocytes, the binding of HBx to JMJD5 facilitates HBV replication via epigenetic alterations in host gene expression. In addition, HBx promotes the formation of ccc DNA by recruiting the transcriptional scaffold, p300; the cAMP response element binding protein CREB; the CREB transcription factor binding protein, CBP; the histone acetyltransferase p300/CBP-associated factor, as well as the histone deactylases HDAC1, Sirt1 [48] and Sirt2 [12]. Once ccc DNA is formed, HBx up-regulates HBV replication, in part, by binding to cullin4-damage specific DNA binding protein (CUL4-DDB1) ubiquitin ligase [64, 65], suggesting that HBx may function, at least in part, at the level of the proteasome. HBx modulates proteasome activity by direct binding to the 26S proteasomal subunit [66], which is responsible for degradation of HBx and several anti-viral proteins. One of the latter is Smc5/6, which is involved in the structural maintenance of chromosomes (i.e., genome stability) and DNA repair [67]. Smc5/6 and HBx bind to the HBV mini-chromosome [67, 68],

resulting in epigenetic changes of virus gene expression. HBx binding to CUL4-DDB1 triggers altered enzymatic activity of the E3 ligase CRL4, which then stimulates the ubiquitination and subsequent proteasomal degradation of Smc5/6 [68–70], thereby promoting virus replication. Other anti-viral systems, such as IFN induced APOBEC3A [50], may also be similarly degraded. In this context, HBV is not very good in triggering innate immunity, which may underscore why there are hundreds of millions of carriers worldwide [71]. As mentioned above, sequestration and reverse transcription of pregenomic HBV RNA in immature nucleocapsids (**Figure 2**) may block the induction of innate immunity. In addition, although HBV replication is exquisitely sensitive to inhibition by IFNs, HBx appears to block IFN expression and signaling [72–74], suggesting that both innate and adaptive immunity could be compromised, thereby permitting virus persistence. Under these circumstances, CLD would continue to damage the liver while being unable to resolve the virus infection. HBx also regulates HBV replication by stimulating the expression of DNA methyl-transferases (DNMTs), which suppresses HBV transcription via DNA methylation [75]. DNMTs also methylate tumor suppressor genes, thereby down-regulating their expression, and permitting the accumulation of mutations and chromosomal instability that contribute importantly to HCC. Thus, HBx regulates the activity of ccc DNA in both positive and negative ways, and in doing so, impacts upon the pathogenesis of CLD. The reason why it is important to regulate the intrahepatic levels of ccc DNA is because when virus antigens are greatly overproduced, they could trigger cytopathic effects (CPE), thereby limiting virus replication. For example, mutations in the preS region of the S gene prevent secretion of surface antigen and complete virus particles, and eventually CPE. Pre-S mutations also promote recycling of viral DNA into the nucleus where it results in increased levels of viral ccc DNA, which potentially promotes virus persistence [76] (**Figure 2**). In transgenic mice overproducing HBsAg, CPE develops and eventually evolves into HCC [77]. Although the latter is not characteristic of HCC pathogenesis among human carriers, it does underscore that selected HBV mutants that may arise during chronic infection potentially contribute to pathogenesis via CPE.

5.2. Oxidative damage and inflammation

Although HBV is not cytopathic, HBx strongly activates NF-κB [78], which promotes the expression of many pro-inflammatory cytokines and chemokines that attenuate virus replication and contribute to the pathogenesis of CLD and HCC. For example, HBx stimulates the expression of IFN inducible proteins, such as the CXC chemokine IP-10 [79] which promotes leukocyte chemotaxis. HBx also stimulates production of interleukin-23 (IL-23) [79], which contributes to the maintenance and expansion of pro-inflammatory Th17 cells. Among others, IL-6 is up-regulated by HBx in a MyD88 manner [80], which indicates that HBx is activating a pro-inflammatory environment via innate immune pathways early on after infection. The repressive effect of IL-6 upon HBV replication is demonstrated by the fact that IL-6 treatment of infected cells results in the loss of HNF1a and HNF4a, both of which bind to ccc DNA. Il-6 also redistributes signal transducers and activators of transcription 3 (STAT3) signaling from ccc DNA to IL-6 target genes [49]. HBx targets up-regulation of IL-18, which up-regulates FasL [81], which in this case blocks the killing of infected cells by CTLs. HBx also up-regulates tumor TNFα [82], which was shown to suppress HBcAg expression [83], thereby inhibiting virus replication. In addition, the pro-inflammatory IL-32 was up-regulated by HBx in a NF-κB

dependent manner [84]. This is not an exhaustive list. Many of these molecules are turned on as a result of HBx stimulating multiple signal transduction pathways in the cytoplasm (in addition to NF-κB), but the bigger question is trying to understand how a non-cytopathic virus is mediating these and other related changes in infected cells.

The fact that HBx plays a central role in HBV replication suggests that intracellular conditions that stimulate HBx activity would also promote the carrier state, which would be evolutionally selected for because it would provide a large window of time for virus to be transmitted to other hosts. In this context, the expression and activity of HBx is stimulated in an oxidative environment, since the addition of anti-oxidants to cells expressing HBx strongly diminish HBx *trans*-activation activity [85, 86]. An oxidative environment (accompanied by oxidative stress of cellular organelles) could be created in the infected cell by virtue of the association of HBx with mitochondria [87]. HBx interacts with the voltage dependent anion channel on the outer mitochondrial membrane, altering transmembrane potential [88], resulting in diminished electron transport, increased free radical accumulation, including elevated lipid peroxidation products [89], release of calcium into the cytosol [55], and under specific circumstances, cell death [90]. Release of calcium into the cytosol, resulted in the activation of the protein tyrosine kinase 2 and Src kinase families, leading to stimulation of ras, raf, mitogen activated protein kinase, and Jun, which stimulate HBV transcription and replication [55]. HBx also induces oxidative stress in the endoplasmic reticulum, which activates the unfolded protein response and expression of pro-inflammatory cyclooxygenase-2 through the activating transcription factor 4 pathway [91]. Free radicals are also characteristic of immune responses aimed at damaging and destroying infected cells that are replicating HBV. In addition, mitochondrial associated HBx induces oxidative stress, which activates selected transcription factors, such as NF-κB, STAT3 and activating protein 1 [86]. However, HBx is also known to block mitochondrial triggered cell death, not only by activation of survival [21, 92] and hepato-protective pathways such as NF-κB that over-ride apoptosis signaling, but also by blocking key caspases and promoting autophagy [93] and mitophagy [94]. The maintenance of mitochondrial and cellular homeostasis by mitophagy acts to attenuate virus induced apoptosis, so that on the one hand, autophagy and mitophagy promote cell survival and virus persistence, while simultaneous mitochondrial damage may contribute to CLD [94].

5.3. HBx and inflammation

In this chronic pro-inflammatory environment, one would expect to see a correlation between HBx staining and the intensity of CLD. In fact, WHx staining has been observed around inflammatory foci in chronically infected woodchuck livers [95], and among human carriers, relatively low levels of intrahepatic HBx staining was observed in patient biopsy samples from people with low grade hepatitis, while intense and widespread HBx staining was observed in patient biopsies from those with advanced fibrosis and cirrhosis [45, 96], suggesting a direct correlation between HBx staining and liver damage. Independent work also showed low levels of HBx mRNA in the livers of patients with mild CLD (e.g., mild hepatitis), and much higher levels among patients with severe lesions in the liver (advanced fibrosis and cirrhosis) [97]. The relationship of HBx expression to disease severity is also consistent with the idea that when the liver regenerates following each bout of hepatitis, fragments of HBV

DNA encoding the HBx region (and sometimes part of the preS/S encoding gene as well) increasingly integrate into multiple regions of the host genome during normal host DNA replication, resulting in increasing accumulation of intrahepatic HBx as CLD progresses. In contrast, the copy number of ccc DNA per cell decreases with regeneration.

The relationship between HBx expression and CLD has been recapitulated in HBx transgenic mice, where the presence, frequency and distribution of HBx in the liver increase with age, as does liver pathology, which progressively develops from hepatitis and steatosis, to dysplasia and microscopic nodules of HCC, and finally to multi-nodular macroscopic HCC with age [98]. In this model, HBx is expressed from its own enhancer and promoter, which is not active until after birth when appropriate transcription factors in the liver begin to appear. HBx expression triggers immune responses in the absence of other HBV gene products, so it is likely that the pathogenesis observed is due to the impact of increasing levels of HBx upon host gene expression combined with immune responses directed against virus infected cells. There is no ccc DNA in this system, just as it is difficult to detect HBV replication among patients with advanced stages of CLD (i.e., cirrhosis). Thus, it is possible that early in chronic infection, and immune responses to virus antigens emanating from ccc DNA templates play an important role in triggering and sustaining immune mediated pathogenesis, but following bouts of CLD and liver regeneration, where the levels of virus replication decrease at the same time that integration of virus DNA fragments increase, pathogenesis appears to be increasingly driven by one or more antigens made from integrated HBV DNA. Although *cis*-acting mechanisms have been postulated to contribute importantly to the pathogenesis of HCC in selected cases, the broadly distributed integration events of the HBx ORF into most chromosomes [99], suggests that the HBx proteins encoded by most integration events promote CLD and HCC in *trans* [47]. In this model, integration of HBV sequences would accumulate in areas of euchromatin and fragile sites much more frequently that at or within specific genes [100].

The model above suggests that targeting ccc DNA in HBeAg carriers with CLD may be an important therapeutic goal to bring about a functional (but not sterilizing) cure due to the presence of integrated virus DNA that express one or more virus proteins. Among anti-HBe carriers with advanced CLD, targeting the much lower levels of ccc DNA may not be effective in preventing progression to cirrhosis and HCC, because at this stage, most of the HBx made probably comes from integrated templates. Under these circumstances, ccc DNA may persist in a transcriptionally inactive form, which is consistent with the absence of HBV DNA in the blood, even after treatment with direct acting anti-viral agents or therapy aimed at stimulating immune responses against virus infected cells [101, 102]. In fact, early work already pointed out that seroconversion to anti-HBe is sometimes associated with the progression of CLD [103, 104], even though later work showed that disease progression was associated with continued replication of HBV DNA carrying one or more mutations in the core gene that blocks production of HBeAg [27]. These mutations were probably selected for during the natural history of infection by immune responses targeting HBcAg [105]. Although these findings suggest that CLD progresses in the liver supporting replication of selected virus mutants, it has also recently been suggested that linear HBV DNA, and not ccc DNA, is the template for integration into host DNA [43], from which one or more virus gene products are made, and contribute to pathogenesis. Thus, persistent inflammation in a chronically damaged liver may result in the development of HCC despite low levels or undetectable levels of virus replication.

6. Conclusions

HBV encodes polypeptides from four ORFs that trigger corresponding immune responses during acute and chronic infections. When these responses are rapid, strong and multi-specific, acute, resolving infection can be achieved. When these immune responses are weak and of limited specificity (against few virus epitopes), the carrier state may develop. Although the pathogenesis of HBV is variable in different hosts, the virus encodes proteins that blunt innate immunity, and as a consequence, adaptive immunity is not triggered at all or to a limited extent. The latter causes liver damage over many years without eliminating the virus. Even though available treatments suppress virus replication, none are curative, and the persistence of viral ccc DNA sustains infection. Production of HBx regulates virus gene expression and replication, but over time, increased integration of HBV DNA fragments encoding HBx results in high levels of HBx expression that epigenetically alter the expression of numerous host genes that up- or down-regulate HBV replication and impact disease activity. For example, HBx activation of AKT decreased HBV replication, but this was accompanied by an inhibition of apoptosis, suggesting that HBx balances HBV replication and cell survival by stimulating signaling that enhance hepatocyte survival at the expense of higher levels of HBV replication [106]. The generation of free radicals by immune responses against virus infected cells, combined with HBx mediated alterations in mitochondrial function, promote HBx activity. These events result in the activation of signaling pathways (e.g., AP-1 and NF-κB) that over-ride apoptosis and/or directly block the activation of critical caspases, so that whether HBx stimulates or block apoptosis depends upon whether the liver is experiencing inflammation and oxidative stress. It also depends upon whether HBx is being expressed in normal hepatocytes, where apoptotic pathways could be triggered, or whether HBx is expressed at high levels in cells where apoptotic pathways are compromised. In addition to being pro-inflammatory, activated NF-κB protects infected cells against immune elimination. Thus, the dichotomy of HBx activity may be a reflection of the environment wherein HBx is expressed. Importantly, the epigenetic mechanisms whereby HBx regulates virus replication also have an impact on cell growth and survival, and many of these same alterations in host gene expression are also hallmarks of cancer [107], which may explain why there is such a high risk of HCC among carriers with CLD [11]. The common denominator is that many of the pathways and molecules that support HBV gene expression and replication also protect infected cells from elimination, and contribute centrally to malignant transformation.

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Conflict of interest

The author declares no conflict of interest.

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Chapter 3

HCC in Elderly Patients. Curative Intraoperative Strategies and Management in Recurrences

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) incidence is growing among general population and especially in elderly patients. Recent development in surgical technique, surgical equipment, interventional radiology, and radiotherapy (hadrontherapy) allows us to use different techniques and approaches in order to treat this cancer. Patients are conventionally considered disease-free after a 10-year recurrence-free period. Commonly, patients remain into a lifelong follow-up and recurrences are treated as they show. In this chapter, we will give description and indications of different curative techniques, especially hepatic resections and Radio-frequency thermal ablation (RFTA). We will also describe and give indications to palliative care techniques such as transarterial chemoembolization (TACE), Selective Internal Radio-Therapy (SIRT), hadrontherapy, and supportive care. The aim of this chapter is to give information to clinicians and specialists dealing with the disease about the most effective approach to treat HCC, taking into account not only biological age, but also "physiological age," performance status, comorbidities, and number of liver operative treatments. This chapter highlights that patients advanced in age are in particular need of a tailored medicine, where benefits are well weighted against invasivity of treatment and its side effects, in spite of assuring the best QoL and survival.

Keywords: HCC elderly, HCC metastases, HCC intervention radiology, HCC surgery, HCC patient management, HCC hadrontherapy, HCC liver resection, HCC cryoablation, HCC laser ablation, RFTA, TACE

1. Introduction

Hepatocellular carcinoma (HCC) is characterized by high clinical and biological variability [1]. Diagnosis and treatment of HCC always require multidisciplinary approaches.

Treatment requires commonly to make a decision between several specific interventions and to choose the one that allows the best risk-benefit ratio for a chosen patient.

Therapeutical approach shall take into account acute cirrhotic impairment risk and patient management experience, thus to avoid iatrogenic prognosis worsening.

Nowadays, patients older than 75 years account for 22% of HCC patients [2]. That is due to treatment and technological advancements which allow to reach an overall survival of decades, if therapy is well pondered; patients are kept in lifelong follow-up and intervention is timed well.

Therapeutical approaches to treat HCC can be divided into surgical approaches, such as major hepatic resection, minor hepatic resection, and wedge resection. Nonsurgical approaches are interventional radiology, chemotherapy, and most recently hadrontherapy. Since people older in age frequently have several comorbidities, often a specific less invasive therapeutical approach is needed.

Age is not a good outcome predictor: fit elderly patients may tolerate radical and invasive approaches, while unfit patients may not [3]. Treatment of older adults must take into account multiple issues related to the condition of aging itself. First of all, patient's frailty, thus invasive approaches are commonly excluded in patients advanced in age; on the other hand, noninvasive treatments are often palliative and do not achieve a satisfactory disease-free survival (DFS) or long-term survival (LTS) [3, 4]. Into this complex scenario, treatment strategies should also consider obstacles to cure the patients either physical or psychological, illness awareness, linguistic or cultural barriers, poverty, depression, and family environment.

Giving indication for or against invasive treatments is arduous in elderly. A decision for intervention shall consider either oncological principles and radical excision on one side or performance status, tolerability of treatment, and actual life expectancy on the other. HCC patients are not only in need for specific treatments, they must also be guided through routine activities in order to ameliorate their own hepatopathic condition, such as lifestyle correction (diet, water and salt assumption, physical activity, and smoking); instructions to the patient himself and to his family for therapeutical adhesion and instruction for early recognition of cirrhotic impairment or therapeutical side effects.

Physiological age is a new fundamental concept which is crucial in evaluating an advance in aged patient's performance status beyond his chronological age, which is still today too often used as a threshold to exclude or include a patient into specific treatment protocols [4].

The aim of this chapter is to give guidelines about management of elderly patients suffering from HCC and to give indications to treat those suffering from HCC as primary malignancy, recurrent illness, or metastatic disease either.

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2. Patient management

2.1. Epidemiology and risk factors

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality. In almost all populations, males have higher liver cancer rates than females, with male/female ratios usually averaging between 2:1 and 4:1 [1, 2].

HCC global distribution varies by region, incidence rate, sex, and also, by etiology. Normally, HCC incidence in female peaks 5 years later than males. Age-specific onset patterns are likely related to differences in the dominant hepatitis virus in population, age at viral infection, and the existence of other risk factors. As for average age at infection, normally, HCV carriers became infected in adulthood, while HBV carriers tend to become infected in childhood [2].

Recently, a significant increase in HCC incidence in hepatitis-free patients was noted; this index had a boost and went from 22% in 2000–2004 to 31% in 2010–2014 (database ITALICA) [5]. Among nonvirus-related hepatopathy, incidence of HCC in Alcoholic fatty liver disease patients (AFLD-patients) remains stable (17 vs. 19%), while a significant incidence increase was seen in Non Alcoholic fatty liver disease Patients (NAFLD-Patients) or in patients suffering from cryptogenic cirrhosis (0.5 vs. 9%) [5].

Often NAFLD-patients are demanding to treat; patients in this group have commonly several comorbidities such as metabolic syndrome; therefore, they need a more accurate and multidimensional clinical evaluation in order to choose the best treatment and achieve the best outcome from their condition.

HCV interferon-mediated clearance, associated with mild to severe fibrosis reduces hepatopathy progression and cirrhosis incidence, thus HCC's incidence reduction in SVR patients is expectable.

HCC hazard in HBV replication-controlled infection is reduced but not abolished, although effective antiviral therapy reduces HCC incidence in HBV- or HCV-positive patients [6].

Sudden HCC recurrence was reported by several papers after Direct Acting Antiretrovirals (DAA) mediated HCV clearance [7]. Other papers have found similar incidence of HCC after DAA-mediated HCV clearance when compared to IFN-mediated SVR but considering an overall 24-month follow-up [7]. HCC incidence after HCV clearance is still not sufficiently evaluated.

2.2. Elderly management and evaluation

More than two-third of patients newly diagnosed with HCC are aged >65 years [8], and this number is expected to increase as the world population ages. Furthermore, there is heterogeneity in the aging process, which further contributes to the complexity of treatment decisions. These factors contribute to age-related variations in treatment patterns and outcomes, potentially resulting in increased likelihood of under- or overtreatment, which can influence both risk of treatment toxicity and survival [9].

Geriatric patients may be extremely complex to treat due to comorbidities that may affect them. Therefore, a clinical evaluation is fundamental to assess the best treatment for each patient.

Aspects that must be considered comprehend not only biological age, HCC stage, and liver status, but also general patient conditions, performance status, and, in particular, individual and familial psychological frame, will to fight against the disease, and treatment tolerability. All these parameters are included into the concept of physiological age which goes far beyond chronological age and considers many crucial aspects of aging which is an extremely individual process.

Since chronologic age alone is a poor descriptor of heterogeneity in the aging process, a systematic and evidence-based way to assess physiological age is needed to guide treatment decisions.

Comprehensive geriatric assessment (CGA) is defined as a multidimensional, interdisciplinary diagnostic process focusing on determining an older person's medical, psychosocial, and functional capabilities to develop a coordinated and integrated plan for treatment and long-term follow-up [10] (**Table 1**).

Important reasons to perform GA in older patients with cancer are detection of unidentified problems and risks for which targeted interventions can be applied and prediction of adverse outcomes (e.g., toxicity, other relevant items such as functional or cognitive decline, postoperative complications); and better estimation of residual life expectancy and lethality of the malignancy in the context of competing comorbidities and general health problems. There is

COMPREHENSIVE GERIATRIC ASSESSMENT
• Medical history and tumor staging;
• Physical examination and physical performance test;
• Karnofsky Performance Status;
• Activities of daily living (ADL);
• Instrumental activities of daily living (IADL);
• Charlson comorbidity score and review of medication;
• Geriatric depression scale;
• Mini mental state;
• Nutritional status (BMI, albumin, haemoglobin, transferrin);
• Social and economic conditions;
• Geriatric Syndromes (Dementia/ Depression/Delirium, Neglect or Abuse, Failure to thrive, Osteoporosis, Falls, Incontinence)

Table 1. Considered parameters in Comprehensive geriatric Assessment.

clear evidence that GA items independently predict OS in a variety of oncology diseases and treatment settings. Poorer OS in older patients with cancer and deficits identified in geriatric domains might potentially be explained by several factors (e.g., increased risk of death resulting from causes other than cancer, increased death resulting from cancer because of less aggressive treatment, or death resulting from complications of cancer treatment) [9].

Patients with risk factors, in particular if older in age, must be lifelong under clinical surveillance for HCC onset. Principal conditions that require normally a twice per year follow-up are advanced cirrhosis, active HCV infection or cleared HCV infection, and HBV-controlled infection. Surveillance is made by liver-US and serum α-fetoprotein assessment. Oncological-marker-only surveillance is not recommendable and since cirrhotic parenchyma is on average poorly explorable, imaging shall be performed by a hepatobiliary dedicated team. Suspect nodules shall be further investigated by CT scan or hepatospecific MRI, which allows to make in the same session either a noninvasive diagnosis or staging when nodule is >10 mm in diameter [11]. Noninvasive diagnosis is cost-effective and a big advantage especially for elderly in poor performance status.

Patients older in age are the cohort that receives the highest benefit in early cancer detection since lower stage HCCs are associated with less invasive interventions, faster recovery, lower mortality rate, and better QoL [4].

Therefore, geriatric patients shall be educated to strictly comply to follow-up timing and to change their lifestyle in order to ameliorate liver function and reduce liver damage.

HCC shall never be considered as a single-cell malignancy: it is a whole organ malignancy; cell transformation is due to liver damage from hepatotropic viruses, toxins, and metabolic syndrome [2, 4]. Even a radical liver resection that cures the single malignancy does not exclude the onset of further lesions and rarely metastases after radical resection is possible [12].

The aim of HCC treatment is to freeze the disease into a chronic stage and to treat lesions as they show. There are several possible treatments that allow in some cases extremely long survival, even in metastatic patients [12].

Management of HCC patients can be extremely complex, so only dedicated multidisciplinary teams shall treat these patients who are regularly discussed into liver units [4].

3. Surgical therapy

3.1. Hepatic resection

Hepatic resection is the gold standard in noncirrhotic liver. In western countries, HCC incidence is raising, mostly due to NAFLD and metabolic syndrome [13].

Patients with these pathologic conditions can develop HCC in the absence of cirrhosis or severe fibrosis [14], although hepatic parenchyma shall not be considered healthy since steatosis is determinable in 50% of patients and steatohepatitis (NASH) in 25% [15, 16]. A

multicentric study confirmed that HCC patients suffering from metabolic syndrome have higher postoperative hepatic failure, mortality, and morbidity rates [15, 16].

Nevertheless, hepatic resection on metabolic syndrome-liver has excellent oncologic effectiveness and leads to long-time survival [16].

In cirrhotic patients, hepatic resection is the first-line treatment for single HCC nodule and preserved hepatic function, strict indications for hepatic resection are serum-bilirubin <1.5 mg/dl and Hepatic Portal-Venous Gradient (HPVG) ≤10 mmHg or platelets ≥100,000 [17].

Resection in patients with light portal hypertension and nonenrollable for liver transplant shall be well weighted against locoregional treatments [17].

Cirrhotic liver resection can be a safe practice in well-selected patients with low morbidity and mortality rates [18]. Selection shall be lead through a global, multiparametric evaluation of the patient and shall pass beyond a dogmatic data interpretation.

All guidelines agree that is needed to select cirrhotic patients for hepatic resection thus to achieve the best outcome, but selection criteria are not universally accepted, the ones suggested from several surgical groups are not based on strong evidence. Therefore, it is necessary to develop a multiparametric evidence-based prognostic score to allow to evaluate a "tailored" operative risk and expected survival.

Tailored-risk evaluation is even more important in elderly patients since aging is a strict-individual process, multidimensional evaluation, and CGA score, in particular, are crucial to assess whether advanced in aged patients can be either enrolled or not for surgery with a deep gap in quality of life and overall survival [4].

Child-Pugh class B patients are routinely excluded from surgery; however, in some cases, satisfying outcome was achieved by performing limited hepatic resections in strictly selected patients, with mild serum bilirubin raise (≤2 mg/dl) and without portal hypertension [19] (**Table 2**).

Laparoscopic or robotic approaches could widen indications to Child-Pugh class B patients due to their little invasivity.

In elderly patients, these approaches are extremely interesting.

HEPATIC RESECTION FOR HCC- INDICATION
• Non-cirrhotic liver (HBV/HCV related liver disease, AFLD, NAFLD);
• Cirrhotic liver: Child Pugh A-B (5-7), MELD <10 Serum bilirubin <1,5 mg/dL HPVG ≤ 10 Platelets ≥ 100.000;
• Tumor staging/ chance of radical resection;
• No severe comorbidities/ ASA I-III;

Table 2. Indications to liver resection in HCC patient.

Hepatic surgery obtains excellent results in elderly patients, even if cirrhotic. Advance age alone is no more a contraindication to surgery.

However, elderly patients, cirrhotic or not, are often excluded from surgery due to comorbidities that rise ASA score and operative risk. Mini-invasive procedures on the one hand make operative time longer and worsen blood-gases control; on the other hand, they allow to spare hepatic parenchyma and shorten hospitalization and recovery [20]. Therefore, patients treated with mini-invasive surgery vs. open achieve a better outcome, especially if elderly, who often suffer longer hospitalizations either physically (reduced physical activity and nosocomial infection risk) and psychologically (depression, confusion, and dizziness) [20].

As reported by a recent meta-analysis, even better outcome is achieved with surgery (open or mini-invasive) vs. transarterial chemoembolization (TACE), which is the most used palliative care technique for HCC, whose advantage remains consistent even in advance HCC, even if vascular invasion is present, so up to stage Barcelona Clinic Liver Cancer (BCLC) stageC [17, 21].

Portal hypertension is often associated with hepatic damage. However, several studies proved that hepatic residual functionality and not portal hypertension affects short- and long-time outcome of hepatic resections [22, 23].

Patients with mild portal hypertension and preserved hepatic functionality can receive limited resections with morbidity, mortality, and OS similar to patients without portal hypertension [19].

HCC frequently develops and spreads through the portal system and that is why several authors recommend performing anatomical resections; these studies prove a better OS and local disease control for anatomic resection vs. wedge resection [24, 25].

More recently, a large Japanese retrospective study (more than 72,000 patients) proved superiority of anatomical resection only for HCC diameter >2 and <5 cm. Superiority is not proven if HCC diameter is <2 cm since portal diffusion risk is very low or >5 cm because other factors influence prognosis [26].

It is also possible to match a parenchyma-sparing surgery with anatomical resection, thanks to subsegmental US-guided resections [27]. This technique with laparoscopic subglissonian or extraglissionan approach is not of common use, due to its technical difficulty and exclusion criteria, that are ascites and moderate to severe portal hypertension. The approach remains interesting and future technical development is possible, especially thanks to robotic surgery. Subsegmental resection shall not be performed for advanced HCC (diameter >2 cm) in order to respect oncologic principles of a radical resection [27]. The procedure allows to spare liver parenchyma, and it may be really interesting for elderly even if cirrhotics with an early-HCC diagnosis.

3.2. Liver transplantation

Liver transplantation (LT) is considered the first-line treatment for cirrhotic patients. LT indications are given following the Milan criteria: single HCC nodules (diameter <5 cm) or less than 3 HCC nodules all <3 cm and in any case nonresectable [17].

A modest expansion to Milan criteria was given by "up to seven criteria," which had achieved satisfactory results in patients without extrahepatic metastases and/or macrovascular invasion. A prospective validation is needed [28].

An increasing number of older patients with end-stage liver disease (ESLD) are evaluated for liver transplantation (LT). In fact, patients aged ≥65 years represent one of the fastest-growing patient populations in LT [29]. The most extreme of these patients, those aged ≥70 years, are associated with several difficult clinical dilemmas. Firstly, advanced patient age is associated with higher risk and poorer outcomes after complex surgical procedures [30]. LT in advanced age patients is associated with increased risk for infection and cardiovascular impairment, increased resource utilization, and lower patient survival [31]. Since the number of adult candidates on the waiting list continues to rise and organ availability remains unable to fully meet this demand, proper organ allocation and utilization are critically important.

Equivalent outcomes can be achieved in elderly recipients and age alone should not be used as a barrier to LT.

Recent data for waitlist registrants on the SRTR registry suggest that <12% of waitlisted patients are aged ≥65 years, but this proportion has steadily increased over the past decade. Continued improvements in care in pre- and posttransplant medicine and surgery suggest that this age group will continue to grow on the waiting list. With this demographic shift in the ESLD, more elderly patients will be considered for LT, and the use of scarce donor livers will need to be addressed because these recipients have a shorter life expectancy compared with younger patients. Despite the shortened lifespan, single-centered reports have shown equivalent posttransplant survival in super-selected patients [32, 33].

Due to physical and psychological impairment, elderly patients are often considered unfit for liver transplantation, since in super-selected groups only satisfying result in LT is achieved, surgical resection remains, for elderly, the first-line approach when performable.

4. Nonsurgical therapies

4.1. Intervention radiology

4.1.1. Radiofrequency thermoablation and microwave thermoablation

Percutaneous radiofrequency thermoablation (RFTA) and microwave thermoablation (MWA) are considered the standard care for patients with BCLC 0-A HCC, who are not eligible for surgical treatment.

Percutaneous ablation techniques are indicated for HCC nodules <2 cm, while nodules with diameter between 2 and 3 cm need to be discussed in a multidisciplinary unit in order to determine an appropriate management plan (**Table 3**). In patients with a single HCC nodule less than 2 cm in diameter, a complete necrosis ratio of 97% is expected [34].

RADIOFREQUENCY THERMO-ABLATION (RTA)- INDICATIONS
• HCC nodules diameter < 2 cm / 2-3 cm in selected cases
• Contraindication for Subglissonian localization, near to vessels or biliary branches due to heat-sink effect
• Contraindication in advanced cirrhosis Child-Pugh > B7 or important ascites

Table 3. Indications to Radio-Frequency Thermal Ablation.

Several randomized studies have documented the superiority of surgical resection over percutaneous ablation techniques in terms of efficacy, while thermoablation has shown lower morbidity, mortality, hospitalization rates, and costs [35].

MWA and RFTA have shown comparable safety and effective results, although MWA seems to have certain theoretical advantages compared to RFTA: shorter procedure, higher ablation temperature, larger area of necrosis, lower probability of biliary duct injury, and reduction in the heat-sink effect through a more uniform heating in the volume of ablation. However, these advantages have not been confirmed in clinical practice. Although EASL guidelines recommend the use of MWA for nodules up to 4 cm, a recent phase II trial, comparing the two techniques in patients with similar mean lesion volumes, showed no significant difference between them in terms of outcome and recurrence ratio [36].

RFTA and MWA can also be safely and effectively performed via a video laparoscopic (VL) approach [37]. VL allows the operator to treat nodules that would normally not be eligible for a percutaneous approach due to nonaccessible locations and allows for hybrid management of patients with multiple nodules (e.g., surgical resection and RFTA on additional nonresectable nodules).

Percutaneous ablation techniques are a precious tool in management of elderly patients with multiple comorbidities.

HCC is often methacronous and new nodules are expected to develop during follow-up after the first tumor. Therefore, it is crucial to perform an appropriate follow-up in patients who have been treated for HCC, in order to detect new nodules at an early stage, so that the least invasive treatment available can be delivered. This is particularly relevant in elderly patients, or those who have already undergone extensive hepatic resection, who might not be eligible for surgery.

Percutaneous ablation is a recommended treatment modality, when indicated, due to its mini-invasive nature, high effectiveness, low rates of adverse events, short hospitalization times, and its relatively few contraindications.

4.1.2. Transarterial chemoembolization

Transarterial chemoembolization (TACE) is a palliative treatment that is routinely used in patients with HCC that are neither eligible for surgery nor for percutaneous ablation, and in

stage BCLC B HCC. TACE is indicated for asymptomatic patients in Child-Pugh class up to B7 and PS ≤ 1 [17] (**Table 4**).

A study found no significant difference in survival following TACE in patients with Child-Pugh Class 8–9 compared to class 7; however, patients with Child-Pugh 8–9 had a significant worse prognosis and more dangerous side effects [38].

TACE is not indicated for patients with signs of HCC vascular invasion, metastases, untreatable ascites, jaundice, thrombosis of a major portal vessel, and HCC nodules >10 cm. In these cases, due to an already compromised liver function, there is a high risk of liver failure and eventually death.

Drug-eluting beads TACE (DEB-TACE) is a more recent variation of conventional TACE (cTACE) that uses embolizing beads eluted with doxorubicin as a chemotherapeutic agent. It has shown overall similar effectiveness, but less systemic side effects compared to cTACE.

Randomized trials have found superior outcomes with DEB-TACE compared to cTACE in patients with Child-Pugh class B and/or PS ≥ 1 [39, 40].

Although contrast-enhanced CT (CECT) or MRI with hepatospecific contrast agent is recommended for TACE outcome evaluation, contrast-enhanced US (CEUS) could be an appropriate alternative in patients with less than four nodules [41].

If imaging follow-up detects residual or recurrent HCC nodules, TACE can be repeated up to three times per nodule. Treatment failure is considered when there are no signs of lesion response, as assessed using the mRECIST criteria, after two treatments or if there is no complete response after three treatments [42]; in eastern countries, different staging criteria, RECICIL, are actually in use [43].

TACE has been proved safe and effective in elderly as well as in younger patients. In particular, a prospective study found that elderly patients suffered from the same complication rates as nonelderly, while effectiveness rates were similar [44].

More important than age is the liver functional status, and the patient's performance status that mostly affects the safety profile of TACE. Therefore, TACE can be an effective palliative treatment able to give benefits in terms of disease control and improved quality of life in elderly patients with HCC.

TRANS-ARTERIAL CHEMO-EMBOLIZATION (TACE)	
Indications	**Controindications**
• Patients not elegible for surgery nor percutaneous ablation; • Child Pugh 8-9; • Performance Status ≤1	• Nodules >10 cm; • Vascular invasion/ Thrombosis of major portal vessels; • Metastases; • Untreatable ascites; • Jaundice

Table 4. Indications to Trans Arterial Chemo-Embolization.

TACE can also be combined with percutaneous ablation, particularly in patients with tumor recurrence within 1 year since the initial treatment, those with tumor diameters of 3.1–5.0 cm, and those with tumor recurrences after initial treatment with thermoablation, where sequential TACE-thermoablation might be the best treatment option [45].

The benefit of this sequential approach is due to the occlusion of hepatic arterial flow by means of embolization before ablation. Furthermore, lipiodol and gelatine sponge particles used in TACE reduce the portal flow around the tumor by filling the peripheral portal vein via multiple arterioportal communications. Therefore, the reduced cooling effect of the hepatic blood flow on ablation-induced thermal coagulation allows the achievement of an enlarged ablation zone which might reduce recurrence rates.

4.1.3. Transarterial radioembolization

Transarterial radioembolization (TARE) is a palliative brachytherapy for HCC. Radioactive substances (I131-lipidol or Y90-beads) are delivered into the tumor by injecting them selectively into its feeding arteries.

This is a complex technique that requires a high-level specialization and has potentially severe side effects such as hepatic, intestinal, and lung toxicity [17]. Therefore, it should only be performed in specialized centers, with high volume activity and experience with this procedure.

Given the fact that TARE has minimal embolizing effects, it can be safely performed even in patients with thrombosis of the portal vein or its branches.

It can be used as a first-line treatment when TACE is not recommended, such as in the case of large or multifocal HCC or if there are signs of portal thrombosis. However, liver function must be conserved (Child-Pugh ≤7, bilirubin ≤2.0 mg/dl, no ascites) [17].

TARE has also been shown to be an appropriate bridge or downstaging treatment in order to meet liver transplantation criteria [17, 46].

Furthermore, TARE can be used as a second-line treatment in patients who did not respond to TACE or who are intolerant to chemotherapy [17, 47].

Mean survival for Child-Pugh class A or B patients who underwent TARE is, respectively, 17.2 and 7.7 months [48, 49]. Mean survival for patients with portal vein thrombosis is 9 months, while for those with intrahepatic portal thrombosis is 17 months [50].

A study has revealed similar results in terms of overall survival (OS) and toxicity between cTACE and TARE in patients with nonresectable HCC [51]; another study has shown a better time-to-progression (TTP) and lower toxicity following TARE compared to TACE [52].

TARE cannot be performed in patients with a pulmonary shunt >20% or if other vascular anomalies may cause irradiation of visceral organs (stomach and intestine) [17].

Indications for this treatment are often controversial and should only be discussed in dedicated multidisciplinary teams. The difficulty in determining the precise indications of TARE is in part due to the lack of cost-effectiveness studies and the fact that its therapeutical equivalence to TACE has only been proved in selected patients. TARE is usually indicated in patients with stage BCLC C HCC, especially those with portal vein thrombosis and preserved liver function.

TARE is usually not indicated in elderly patients, who often have a compromised liver function, and therefore, risks of liver failure and death are high. TARE can be performed in elderly with good performance status and liver functionality as a second-line treatment in patients with treatment failure following TACE.

TARE has not been shown superior to sorafenib in treating advanced HCC; therefore, sorafenib could be a safer treatment in elderly patients who can tolerate chemotherapy [53].

4.1.4. Other

4.1.4.1. Percutaneous ethanol injection (PEI)

PEI induces cell necrosis through dehydration, protein denaturation, and small vessel disruption. It is not often used since it can only be performed in lesions <2 cm and it has a higher recurrence ratio than percutaneous ablation. It has indication only in lesions that are not considered safe for ablation due to their localization [54].

Compared to PEI, RF has shown better outcomes in terms of overall survival, survival at 1, 2, and 3 years, and cancer-free survival at 1, 2, and 3 years. This is probably due to the better performance of RF in terms of complete necrosis of the lesion and the low percentage of local recurrence [54].

RF requires fewer treatment sessions and shorter hospitalization than ethanol injection: although the quality of life of these patients was not evaluated, there was a decrease in hospitalization rates [54].

4.1.4.2. Cryoablation

Cell death with cryoablation is different than that with thermal ablation. The freezing process results in both intracellular and extracellular ice formation, both of which can result in cellular death, but by different mechanisms. Since the ablation zone is reperfused after the ice ball melts, the result is a rapid release of cellular debris into the systemic circulation. This probably explains the systemic complications of cryoablation (i.e., cryoshock) that are rare with heat-based ablation. Thermoablation is the preferred ablation method for treating HCC in patients with cirrhosis because of the increased risk of bleeding and of disseminated intravascular coagulation-like reaction (called cryoshock) associated with cryoablation [55, 56]. Therefore, although many studies have shown that small-volume cryoablation is feasible in patients with cirrhosis and HCC, it is difficult to justify the additional risk of cryoablation in these patients when viable heat-based alternatives are available [55].

4.1.4.3. Laser ablation (LA)

The term laser ablation refers to the thermal tissue destruction by conversion of absorbed light (usually infrared) into heat. Infrared energy penetrates tissue for 12–15 mm in depth; heat is conducted beyond this range thereby creating a larger ablation area. Optical penetration has been shown to be increased in malignant tissue compared to normal parenchyma [57].

Local tissue properties, in particular perfusion, have a significant impact on the size of the ablation zone. Highly perfused tissue and large blood vessels act as a heat sink, since infrared energy is absorbed by erythrocytic heme and transported away from the target area. This phenomenon makes normal liver parenchyma relatively more resistant to LA than tumor tissue and this is the rationale for using hepatic inflow occlusion techniques such as arterial embolization (TACE) in conjunction with laser therapy [57].

Light transmission into tissues and the size of the ablation zone increase with higher laser power, as does the local tissue temperature reached during ablation, with consequent higher risk of overheating and carbonization of the adjacent normal tissue.

The use of water-cooled laser application sheaths allows the use of a higher laser power output while preventing carbonization [58]. When using multiple water-cooled higher power fibers, ablation zones of up to 80 mm diameter can be obtained.

Major complications of LA are liver failure, segmental infarction, hepatic abscess, cholangitis, bile duct injury, and hemorrhage. The technique is considered safe by rates of 1.8% for major complications and a mortality rate of 0.1% [59] and can also be used safely in elderly patients with advanced liver disease up to Child–Pugh class B [57]. Tumor seeding after percutaneous biopsy and ablative therapies is a well-known phenomenon, but it has rarely been reported following laser ablation [57].

A recent study compared LA and TACE in patients with a single large HCC and found a significant superiority in multifiber-LA vs. TACE in terms of recurrence rates, especially in nodules >4 cm, while OS was similar between both groups [60].

Ablation size is critical to predict outcome; patients with lesions >6 cm or with multifocal disease (more than five nodules) are usually managed with other treatment modalities.

LA can be used with a curative intent only in patients with early-stage HCC. In this setting, it has shown similar outcomes compared to RFTA when treating nodules <3 cm [57, 60].

In patients with advanced local HCC, LA should only be used as a palliative treatment. The use of laser ablation is not currently extensively adopted for the treatment of HCC, but given the promising outcomes shown in recent studies and the expected technical advancements, it could become an increasingly more important treatment modality for HCC in the near future.

4.2. Chemotherapy

Systemic therapy is recommended for HCC patients in stage BCLC-C with conserved liver functionality (Child-Pugh A), good performance status, advanced disease, and/or extrahepatic diffusion. Systemic therapy is also recommended for patients with progressive HCC after locoregional treatments or HCC with vascular invasion not enrollable for other local treatments [17].

Target therapy with sorafenib proved to give survival benefits versus either placebo or cytotoxic and hormonal therapy [61].

Observational studies suggest that sorafenib administration in Child-Pugh B patients is as safe as administration in class A patients [62].

Recently, RESORCE trial showed survival benefit in regorafenib administration vs. placebo for HCC patients that went to tumor progression after sorafenib administration; all patients had tyrosine kinases-associated adverse effects [63].

Cytotoxic chemotherapy, such as doxorubicin or FOLFOX4 scheme, can be considered in patients with conserved liver functionality and after that sorafenib therapy has suspended for adverse effects [17] (**Table 5**).

Adverse effects of sorafenib especially dermatological, hypertension, or diarrhea in the first month of treatment are a frequent cause of treatment failure; it was proved that half-dose administration after adverse effects is associated with survival benefits [64].

In some cases, for fit and super-selected patients, intolerant to sorafenib, in case of oligometastatic disease, a different disease management can be done. Mini-invasive surgical therapy along with intervention radiology may be able to remove several metastases and treat them as they show; once the primitive tumor is surgically resected, a chronic metastatic disease can be surgically controlled with survival benefits [12]. Only case reports on this field have been published, but these authors believe that more research shall be done with multicentric clinical trials to prove what has been shown only in case reports.

Elderly can hardly ever be treated with surgical therapy due to their frailty and low PS even if in many cases, biological age does not correspond to chronological age. Sorafenib showed similar results in terms of safety and effectiveness in elderly and younger HCC populations. When administering systemic therapy, careful baseline evaluation is needed for patient's selection in elderly population, including discussion about antiplatelet therapy discontinuation, and caution in PS ≥ 1 patients, as well as active management of toxicity.

Asthenia and bleeding are more frequent in the elderly. The higher frequency of bleeding is explained by concomitant antiplatelet treatments, and major asthenia is frequent in PS ≥ 1 elderly patients [65].

A multidimensional evaluation is crucial for elderly patients and also in advanced HCC, and decision to start systemic therapy shall be made by experienced and dedicated units.

Adverse Effects of Sorafenib
• Dermatological toxicity;
• Hypertension;
• Diarrhea;
• Nausea and vomiting;
• Haematological toxicity

Table 5. Most common adverse effects of Sorafenib.

4.3. Best supportive care

Palliative treatments and supportive care aim at ameliorating patients QoL and at giving relief by symptoms. Terminal-stage HCC may have several symptoms associated with liver dysfunction due to cirrhosis, such as ascites, esophageal hemorrhage, and hepatic encephalopathy. Abdominal pain and asthenia are common.

Paracetamol and opioids are the safest drugs for pain control in hepatopathics; Non Steroideal Antinflammatory Drugs (NSAIDs) shall be avoided due to hemorrhage risk, kidney dysfunction risk, and resistant ascites development.

Radiotherapy is effective in pain control due to bone metastases; control results complete in 50% of patients and partial in 80–90% [66].

Percutaneous cementoplasty is effective in controlling HCC vertebral metastases' pain [67]. Brain metastases are rare and selected cases can be treated with stereotaxic radiotherapy [68].

Malnutrition and cachectic-state is common in end-stage oncological patients, in particular if affected by noncontrolled cirrhosis which enhances weight loss and muscular tissue loss.

Nutritional state assessment is important in HCC patients and was observed that prognostic nutritional index can predict survival expectancy in HCC patients [69].

4.4. Frontiers in palliative treatment of HCC: hadrontherapy

Hadrontherapy or heavy charged particle therapy (CPT) is one of the newest palliative treatments available against HCC.

Hadrontherapy technology is based on charged particles (carbon ions), which accelerated by cyclotrons or synchrotrons are conveyed into a beam to irradiate the tumor. Different from X-rays, charged particles have a sharp Bragg's peak which is even sharper than the one of protons; therefore, they release a great part of their energy at a specific level of tissue penetration which is proportional to their kinetic energy.

This technology allows to concentrate cellular damage into a very small area; therefore, CPT has higher tumor control probability (TCP) and relative biological effectiveness (RBE) than other radiotherapy techniques, it can also reduce organs at risk (OAR) and nonmalignant tissue complications probability (NTCP) [70].

Due to the physical properties of charged particles and, in particular, the possibility to generate a heavy concentrated damage, hypofractioning is possible with good results in terms of adverse effects.

Clinical trials on CPT are still running; first data are hopeful; in a Japanese study that used CPT in nontreatable HCC, a 5-year local control rate was 81% and survival was 33%; results are similar to those for proton therapy with 20 fractions, but by using a total of 4 fractions in 2 days [71]. Good results are also obtained with difficult to treat porta-hepatis HCC [69]. As for HCC metastases, they can be treated with a 50.4 Gy irradiation in 12 fractions [12, 71].

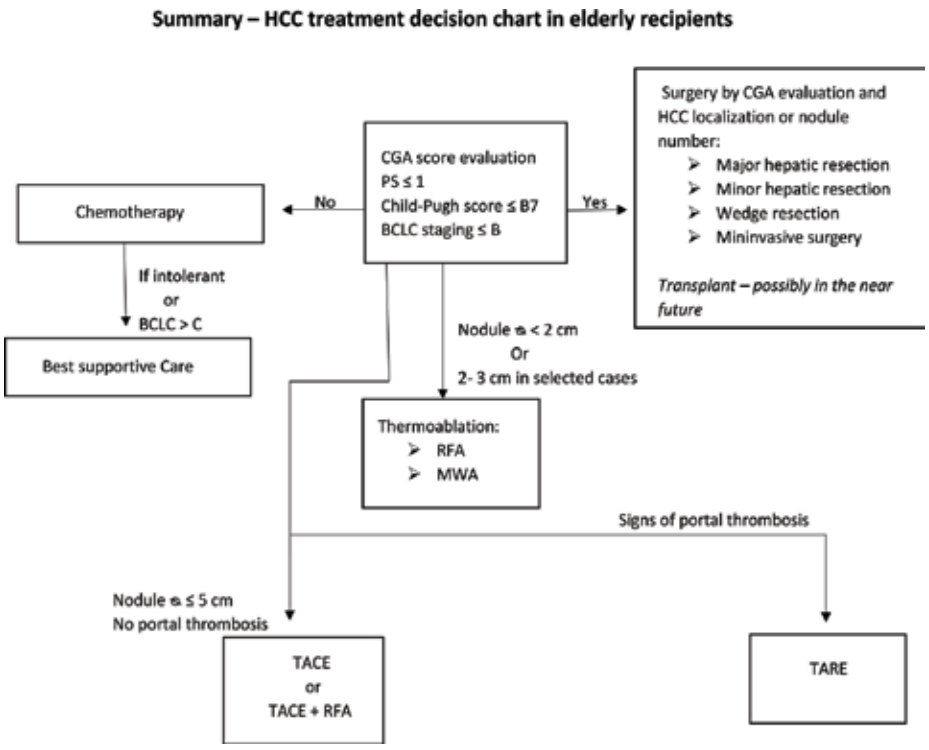

Figure 1. Summary—HCC treatment decision chart in elderly recipients.

These treatments are still experimental but results of trials until now are encouraging, in the next future hadrontherapy may be one of the pillaRs of advance HCC treatment.

Hadrontherapy may be extremely interesting as a treatment also for elderlies, since it has little adverse effect and thanks to hypofractioning and noninvasivity; in the future, it may become a treatment of choice for difficult-to-treat HCC in elderly and for metastatic disease. It may ensure long-time tumor control and good QoL even in people advanced in age; the only issue of this treatment is its high cost-effectiveness ratio, even though cost-effectiveness trials for CPT are not still published a CPT apparatus costs around US$ 200 million; only three carbon ion centers are available in Western Europe, seven in Asia (Japan and China), and none in US [65] (**Figure 1**).

5. Conclusion

The aim of this chapter is to give information and indications about the most recent operative and nonoperative existing techniques to treat HCC. Focus on older adults' case evaluation is of extreme importance; because lifespan enlargement will produce, in next decades, a sharp rise in HCC incidence among elderlies [2].

It is strongly believed, by this multidisciplinary team, that early diagnosis is the key for HCC eradication in general population and in particular in elderly: dealing with a lower-staged cancer allows to use both less invasive and more radical treatments. Patients would then suffer less hospitalization time; would have faster recovery and lower infection risk [4]. Hospitalization time and subsequent infections are the most common cause of death for hospitalized elderly patients. Early-HCCs grow slowly when they develop in elderlies and when removed surgically or with interventional radiology mean (RFTA or MWA) recurrences appear after a sufficient time latency thus to make it possible to chronicize the disease and allow to reach survival rates not different from general population [4].

The elderlies are a very heterogenic population; therefore, this kind of patients cannot be treated with a standardized protocol, but a tailored approach is needed. Each patient has its own comorbidities that must be taken into account; moreover, aging itself is an extremely individual process and different patients may have wide differences in performance status and therefore different treatment indications. Life expectancy, comorbidities, liver functionality, cancer progression, patient's therapy compliance, psychological status, and performance status shall be all taken into account when cases are discussed into multidisciplinary teams in order to assure the best treatment, and therefore, the best OS and QoL.

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Section 3

Surgical Resection of Liver Cancer

Chapter 4

Surgical Resection in HCC

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is a deadly disease. Its incidence is rising worldwide without significant improvement in survival in spite of improving therapies. A wide array of treatment options for HCC exist and include surgery, catheter-based therapies, radiation and systemic therapy. These modalities are often used in combination for optimal management in a multidisciplinary approach. Surgical resection remains one of the only curative therapeutic options for HCC, although it is indicated in select patients with localized disease. Herein, we cover the role of surgical resection in the management of HCC, reviewing the perioperative and operative considerations, in addition to highlighting the advances in minimally invasive surgery and novel navigation technologies.

Keywords: hepatocellular carcinoma, liver cancer, surgery, minimally invasive, multidisciplinary

1. Introduction

Hepatocellular carcinoma (HCC) is the second most lethal malignancy worldwide [1]. Despite the advent of effective antiviral drugs to eradicate hepatitis C infection, the prevalence of HCC is projected to increase secondary to increasing rates of fatty liver disease from diabetes and the obesity epidemic [2]. Unfortunately, there has been little to no change in the survivability of HCC over the last three decades [3] in spite of the increasing array of therapeutic options, leaving much room for improvement. The armamentarium for managing HCC is wide and includes surgical resection, orthotopic liver transplantation (OLT), ablative techniques using ethanol (percutaneous ethanol injection, PEI), microwave (MWA) or radiofrequency (RFA), catheter-directed transarterial chemoembolization (TACE) or radioembolization (TARE),

external beam radiation therapy in the form of stereotactic body radiation therapy (SBRT) or proton beam therapy (PBT), systemic targeted small molecule tyrosine kinase inhibitors, check-point inhibitor immunotherapy and investigational agents. These modalities are often used together in a multidisciplinary approach.

Surgical resection, or partial hepatectomy (PH), is a potentially curative surgical treatment option for up to 15–20% of patients with HCC. The primary objective of PH is to remove the HCC with an adequate margin, while preserving as much functional liver parenchyma to avoid post-resection hepatic failure. With improvements in preoperative assessment, patient selection, surgical and anesthetic techniques, intraoperative ultrasound, PH for HCC is now routine and safe. Operative mortality has been reduced to less than 5% with a 5-year overall survival of 60–75%.

2. Preoperative considerations

Several factors are considered in determining the eligibility for PH, including the patient's health status (e.g. age, ECOG PS), tumor-specific factors (e.g. extent and tumor biology), and the reserve of the liver remnant. Determined by the degree of liver dysfunction and the size of the postoperative liver remnant. While there is no strict age limit, one must consider the liver's regenerative capabilities in elderly patients, and the patient's ability to tolerate the physiologic consequences of portal pedicle clamping and acute hemorrhage on their cardiopulmonary system. In addition, patients undergoing a minimally invasive approach must also be able to endure the effects of the pneumoperitoneum and reverse Trendelenburg positioning on their physiology.

Several different clinical staging systems exist to stratify patients according to prognostic variables [4]. One of the most commonly used is the Barcelona Clinic Liver Cancer (BCLC) system which incorporates tumor size, number of nodules and hepatic function as classified by the Child-Pugh score [5]. The system classifies patients into early, intermediate, advanced and terminal stages and proposes recommended treatment strategy. According to this staging system, only stage 0 or early stage patients with small tumors are recommended for surgical resection or liver transplant.

However, many view the BCLC criterion for resection to be restrictive. For patients with large tumors (beyond any down-staging or expanded OLT criteria) who are ineligible for OLT, PH is the only potentially curative treatment. With improvements in perioperative management, preoperative morphological assessment and manipulation of the future liver remnant, PH for large HCC has been safely performed with good oncologic outcome [6, 7]. Therefore, large tumor size alone is not a contraindication to PH, rather factors such as multiple or bilobar tumors, extrahepatic metastasis, involvement of the main bile duct, portal venous or other macroscopic vascular invasion, and portal hypertension are all relative contraindications to PH. When clinically not evident, portal hypertension can be evaluated by measuring the transjugular intrahepatic portosystemic gradient (PSG). PSG values greater than 10 mmHg are indicative of significant portal hypertension and these patients must be approached with caution.

85–90% of patients with HCC have concomitant liver dysfunction. It is critical to account for the degree of liver dysfunction in addition to the patient's overall functional and nutritional status. Patients with liver disease are often malnourished with diminished performance status and comorbid conditions. To help stratify clinical liver dysfunction, patients are classified by the Child-Turcotte-Pugh (CTP) score and the Model for End-Stage Liver Disease (MELD) system. These two systems classify patients based on physical exam and laboratory data, with increasing scores associated with higher overall surgical risk. In general, patients with CTP score up to B7, MELD score <9 without significant portal hypertension can be considered for PH. Patients with more severe liver dysfunction and HCC can be considered for OLT if they meet specific criteria [8, 9].

Assessment of the hepatic function and future liver remnant (FLR) is important for patient selection prior to surgical resection [10]. The volume of the FLR and the regenerative capacity are key predictors of postoperative morbidity. Several laboratory tests have been used to evaluate hepatic reserve in cirrhotic patients including assessment of clearance of indocyanine green, sorbitol and 99mTc-galactosyl serum albumin scintigraphy [11]. Preoperative volumetric analysis can be performed with 3D computerized tomography volumetry [12]. To minimize the chance of post-hepatectomy liver failure, data suggest a liver remnant to be at minimum >20% of preoperative liver volume in a normal functioning liver, >30% for patients who have undergone >3 months systemic chemotherapy and >40% in those with advanced liver disease [13, 14].

Several techniques for preoperative optimization of the FLR exist including portal vein embolization (PVE) and the associated liver partition with portal vein ligation for staged hepatectomy (ALPPS) [15]. Initially developed in 1986, PVE results in atrophy of the embolized segments and compensatory hypertrophy of the perfused segments [16], within approximately 4–6 weeks, with at least >10% growth of the FLR predicting adequate regeneration post-PH. PVE has been shown to reduce the rate of postoperative complications in select patients with chronic liver disease [17], and can also be used safely in patients undergoing concurrent chemotherapy for colorectal metastases. One study demonstrated improved prognosis after PH in patients with impaired hepatic function [18].

ALPPS was developed in 2007 to induce liver hypertrophy in patients planned for extended liver resections with marginal FLR. A two-step operation, the initial data demonstrated it to be quite effective with rapid hypertrophy [15], however, it has not gained wide acceptance secondary to significant morbidity and mortality and the need for larger scale studies [19–21]. However, there are more recent reports of "mini-ALPPS" where the procedure is performed minimally invasively and with limited peripheral division of the parenchyma.

3. Surgical considerations

3.1. Surgical anatomy

The surgical anatomy of the liver is based on Claude Couinaud's classification system and further refined in the Brisbane 2000 Terminology of Liver Anatomy and Resections (**Figure 1**)

[22]. In this classification, the liver is divided into first, second and third order divisions based on internal anatomy rather than surface landmarks. First order division splits the liver into a right and left hemiliver along Cantlie's line, a plane extending from the middle of the gallbladder fossa to the center of the inferior vena cava. Second order divisions split the hemilivers into two respective sections or sectors, the medial and lateral sections/sectors on the left and anterior and posterior sections/sectors on the right. The third order division divides each section/sector into two segments, constituting the 9 individual hepatic segments defined by Couinaud. In general, each segment has a unique vascular inflow, outflow and biliary duct enabling segments to be removed without damage to other segments.

The proper hepatic artery and portal vein bifurcate prior to the hilum of the liver and form the right and left hepatic artery and portal vein which supply the right and left hemiliver. Joined by the biliary duct, the portal triad generally runs centrally within hepatic segments. The right hepatic artery enters the parenchyma soon after branching while the left has a longer extrahepatic course. In contrast, the three hepatic veins run between section/sectors in three portal scissurae. The right hepatic vein drains directly into the inferior vena cava (IVC) while the middle and left hepatic veins often form a common trunk prior to entering the IVC.

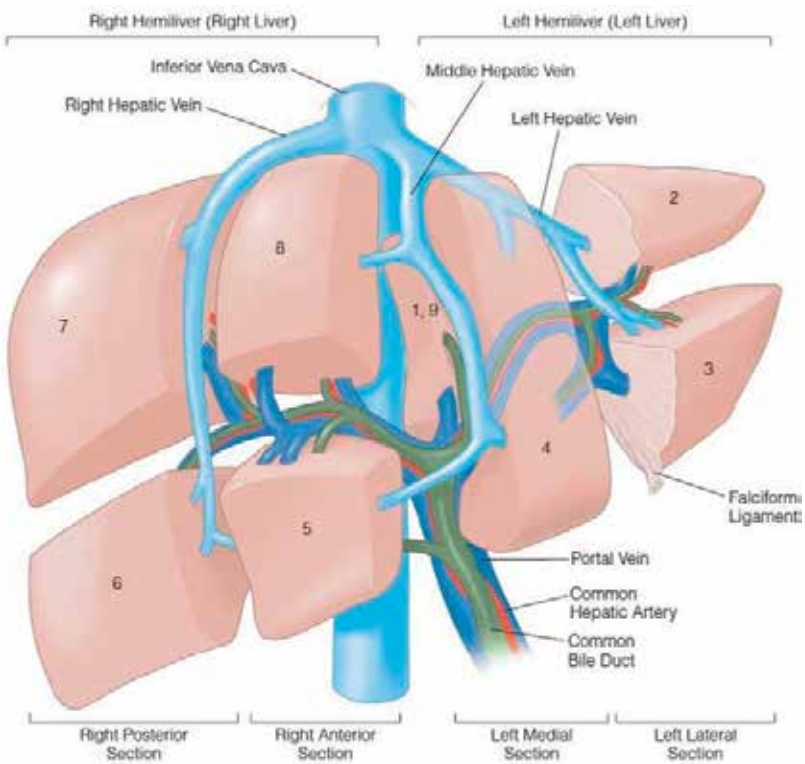

Figure 1. Schematic of liver anatomy separating the parenchyma into 9 anatomic segments. Each segment has unique blood supply and biliary drainage. Source: Cho, Fong. Hepatic Resection. In: Ashley SW, editor. Scientific American Surgery. Hamilton: Decker. 7th ed; 2014. pp. 1094–1114.

The liver is encapsulated by a fibrous capsule, known as Glisson's capsule. The capsule envelops the portal triads as they enter the liver parenchyma which makes it identifiable on intraoperative ultrasound. Furthermore, the dense capsule allows for control of the portal triad during dissection and enables pedicle ligation.

3.2. Anesthetic considerations

Some important perioperative anesthetic considerations should be accounted for to increase the safety of hepatectomy. To minimize the possibility of major intraoperative hemorrhage, the central venous pressure should be maintained at less than 5 mmHg to reduce the intrahepatic venous pressure. This is achieved using various anesthetic maneuvers and agents such as IVF restriction, and administration of isoflurane, fentanyl, mannitol, and cisatricurium. For open hepatectomy, the patient can be placed in slight reverse Trendelenburg position if pressures allow and switched to Trendelenburg position if there is significant hemorrhage with hemodynamic derangement to increase cardiac output and maintain end-organ perfusion. For laparoscopic/robotic hepatectomy, the patient is placed in reverse Trendelenburg position for a caudal approach which improves visualization of the vasculature, and the pneumoperitoneum creates a tamponade effect on the hepatic veins, which aids in limiting hemorrhage. Adequate vascular access should be obtained using large bore IVs, with appropriate invasive hemodynamic monitoring using A-line. Blood products should be readily available and resuscitation of operative blood loss should be with an appropriate combination of crystalloid, albumin and blood product as necessary. End-tidal CO_2 is measured to monitor for CO_2 embolism in the laparoscopic/robotic approach.

4. Operative technique

Resections are either "anatomic" or "non-anatomic". Anatomic resection defines a resection that obeys Brisbane divisions and is preferred for malignant disease because it has been found to lower rate of positive margins, decrease regional recurrences and improve surgical outcome. Non-anatomic resection refers to parenchymal transection that does not respect segmental planes and is typically used for debulking procedures, benign tumors or when trying to preserve remnant parenchyma. Achieving a microscopic margin negative (R0) resection is paramount to reducing local recurrence. 1 cm surgical margins have historically been considered standard, but narrower margins have been safely demonstrated [23].

There are six standard, anatomic hepatic resections as defined by the Brisbane classification (**Figure 2**). Right hemi-hepatectomy consists of surgical resection of segments V-VIII and left hepatectomy includes segments II-IV and occasionally segment I. In an extended right hepatectomy or a right trisectionectomy/trisectorectomy, segments IV-VIII, and in an extended left hepatectomy or a left trisectionectomy, segments II-IV, V and VIII are resected. A left lateral sectorectomy involves resection of segments II-III and a right posterior sectionectomy includes segments VI-VII. Segmentectomies denote resection of any individual segment.

The common principle of anatomic hepatectomies involves parenchymal transection after both vascular inflow and outflow have been controlled. Given that each hepatic segment has

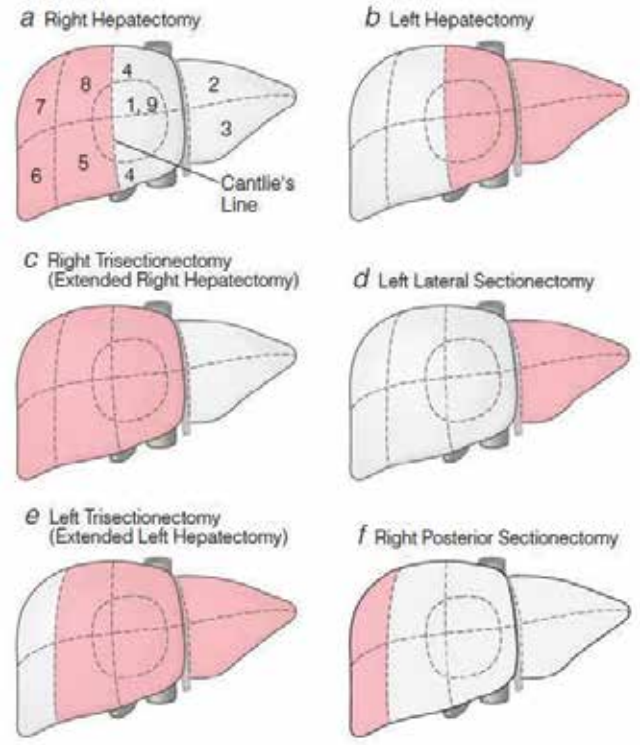

Figure 2. Schematic illustrations of the standard hepatic resections as labeled. Source: Cho, Fong. Hepatic Resection. In: Ashley SW, editor. Scientific American Surgery. Hamilton: Decker. 7th ed; 2014. pp. 1094–1114.

their unique vascular inflow and outflow, each segment can be safely excised without damage to surrounding hepatic segments. Intraoperative ultrasonography is used routinely for identification of the vascular structures, evaluation of tumor location, extent and relationship to the surrounding vasculature.

After initial laparoscopic inspection excludes unresectable disease (in selected cases), the incision is made. In an open conventional approach, appropriate incision and exposure is critical to safe hepatectomy. There are several incisions used including the bilateral subcostal (Chevron), right/left subcostal, J-type or the inverted Y (Mercedes) incision.

Once the liver is mobilized by dividing ligamentous attachments, careful inspection, palpation and ultrasound examination are performed to evaluate for any missed tumors. Arterial aberrancies are identified and portal triad inflow is controlled with sutures and clips or staple ligation. The corresponding hepatic vein is isolated and ligated. Parenchymal transection is performed along the line of devascularization. Different techniques for parenchymal transection exist, varying from clamp-crushing, waterjet, monopolar/bipolar cautery, radiofrequency ablative devices, bipolar vessel sealing devices, ultrasonic dissection devices to staplers. The clamp-crush technique is rapid and has been associated with lower rates of blood loss compared to other methods [24]. Once the resected segment is removed, hemostasis is obtained

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with sutures, clips, argon beam coagulator and application of various hemostatic agents. Biliary leaks are controlled with clipping and suture ligation. Prior to abdominal closure, drains are placed if there is an infected operative field or if a biliary reconstruction is performed [25].

5. Minimally invasive hepatectomy

5.1. Laparoscopic-assisted partial hepatectomy

Although established as a safe and beneficial approach for numerous intra-abdominal operations, laparoscopic techniques were slow to be adopted for liver surgery for several reasons [26]. Concerns over technical feasibility of vascular dissection and control, organ mobilization, parenchymal dissection and management of intraoperative complications were prohibitive. Furthermore, it was unknown if port-site seeding, inadequate margins and poor oncologic outcomes would be more common in the minimally invasive approach.

The benefits of laparoscopic liver surgery are numerous. In addition to the generalized benefits of laparoscopic surgery including a more rapid functional recovery, smaller incisions which reduce the incidence of surgical site infections and postoperative pulmonary complications, there are additional advantages specific to laparoscopic liver surgery. Steep Trendelenburg positioning reduces intrahepatic venous pressure and the pneumoperitoneum exerts tamponade effect on vasculature leading to reduced intraoperative blood loss. Laparoscopy creates a caudal-cranial surgical view which affords improved visualization of major vascular structures compared to the ventral-dorsal angle of visualization of an open hepatectomy. For cirrhotic patients, small laparoscopic incisions avoid disruption of abdominal wall collaterals and the constraint on fluid shifts in a laparoscopic partial hepatectomy can decrease the incidence of liver-related complications. Minimally invasive hepatectomy also results in less adhesion formation which facilitates additional surgery in the future.

There have been numerous studies to date demonstrating the safety and efficacy of laparoscopic liver surgery. In 2009, a worldwide experience of 127 series including 2804 cases of laparoscopic partial hepatectomy demonstrated comparable 5-year overall survival and disease free survival compared to open hepatectomy [27]. Half of these cases were done for malignant disease with greater than 80% of resections boasting negative surgical margins. In 2015, a randomized control trial was published demonstrating safety and feasibility of laparoscopic liver resection with reduction in length of stay and intraoperative blood loss compared to open hepatectomy [28]. Numerous systematic analyses have substantiated these data, demonstrating that the laparoscopic partial hepatectomy is associated with decreased intraoperative blood loss, shorter length of hospital stay, and decreased number of positive resection margins. Overall, there were consistently fewer complications found in the laparoscopic group in these reviews [29]. A case–control propensity matched studies also found no difference in 1-, 3-, and 5-year overall survival and disease-free survival [30]. The National Surgical Quality Improvement Program database was evaluated to compare short-term outcomes among patients undergoing minimally invasive partial hepatectomy. Over 3000 patients were include in the study

and it demonstrated lower postoperative morbidity and shorter length of stay compared with patients undergoing open liver resection [31].

Specific to the treatment of HCC, the safety and efficacy of the laparoscopic approach has been evaluated in several meta-analyses and propensity score analyses. These studies demonstrated the equivalent or superior perioperative outcomes of laparoscopic compared to open resection [32, 33]. In a propensity score analysis, the overall and disease-free survival were similar and for the secondary outcomes, the laparoscopic group had shorter hospital stay, lower morbidity, with fewer transient liver failure and wound complications, and a larger tumor margin [34].

Multiple meta-analyses and case control series were reviewed and analyzed at the second international conference for laparoscopic liver resection in Morioka in 2014. Minor resections were validated as standard practice in the assessment stage, while major or complex resections were considered to be in the exploration stage, with incompletely defined risks. The Jury at Morioka made strong recommendations for higher quality studies including registries to define the role and benefits of laparoscopic major hepatectomy.

Patient selection is critical to ensuring safe laparoscopic partial hepatectomy. Although is technically feasible, resection of lesions in right posterior sections or the hepatic dome can be challenging and should be approached with caution. The patient is placed in the supine position and securely fastened to the table to allow for safe intraoperative repositioning. Generally, five ports are required for laparoscopic resection including two 12 mm and three 5 mm ports. Port placement is dependent upon laterality of the lesion as shown in **Figure 3**. Some surgeons advocate using a hand access port to assist with intraoperative manipulation, intra-corporeal suturing as well as serve as the specimen removal site.

5.2. Robotic-assisted partial hepatectomy

Further advances in surgical technology has created new opportunities in minimally invasive liver surgery. Robotic surgical systems offer unique advantages to the liver surgeon that enhances the minimally invasive approach. There are several key improvements on the robotic surgical system including a camera with optics providing a 3-dimensional stereotactic visual field. In addition, the instruments allow for seven degrees of freedom in their motion, providing easier suturing for hemorrhage control. There is no fulcrum effect on the body wall of the patient as in laparoscopic surgery, and it has been associated with reduction in surgeon fatigue compared to the laparoscopic approach.

Similar to laparoscopic partial hepatectomy, the patient is placed in the supine position and in steep reverse Trendelenburg position. The table is tilted with right side up approximately 25 degrees for right-sided resections. Five ports are placed including four robot-controlled ports and one assistant port (**Figure 4**). The ports are placed based on the laterality of the resection. In general, for a right-sided hepatectomy, the camera port is placed to the right-side of midline. Once the ports have been placed, the robot is docked from the cephalad position (**Figure 5**). Intraoperative ultrasound is critical to establishing vascular anatomy and defining oncologic planes of resection. After vascular control and establishing the line of transection, parenchymal transection is performed using one of many published techniques [35].

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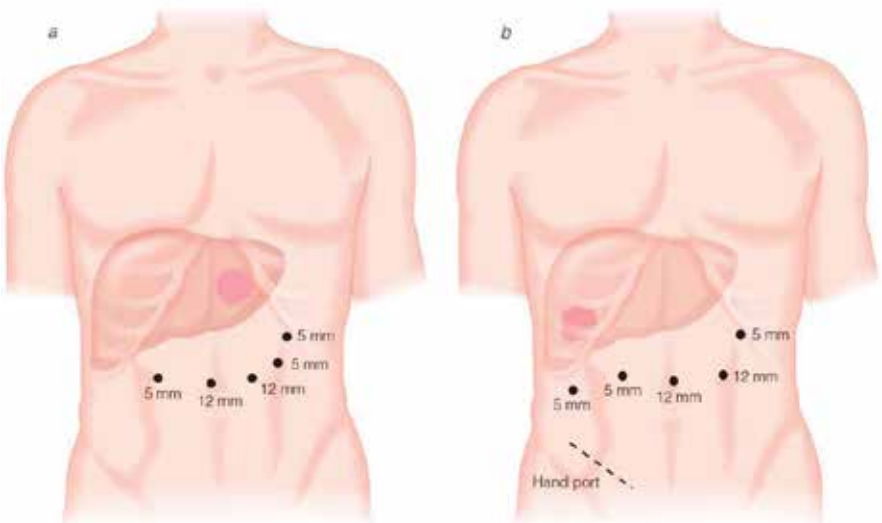

Figure 3. Suggested port placements for laparoscopic left lateral sectionectomy (a) and hand-assisted laparoscopic right hepatectomy (b). Source: Cho, Fong. Hepatic Resection. In: Ashley SW, editor. Scientific American Surgery. Hamilton: Decker. 7th ed; 2014. pp. 1094–1114.

Several large case series have been published demonstrating the success of robotic liver resection [36, 37]. The first large case series of 70 patients included 38.5% major liver resections without any mortalities [36]. An early systematic review of the literature demonstrated safety and feasibility of the robotic technique, with conversion to open rate of 4.6% and complication rate of 20.3% [38]. In 2018, an international, multicenter retrospective review of robotic liver surgery was published specifically evaluating long-term oncologic outcomes in patients with primary hepatobiliary malignancies after a median follow up of 75 months [39]. This study demonstrated comparable outcomes between robotic, open and laparoscopic liver surgery with 3-year overall survival of 90% for HCC. The majority of the cases were non-anatomic resections with an R0 resection achieved in 95% of HCC resections, 68% in cholangiocarcinoma and 82% in gallbladder cancer.

Minimally invasive approach to liver surgery, both laparoscopic and robotic-assisted, have their share of limitations. An important potential complication associated with the establishment of pneumoperitoneum and laparoscopic liver surgery is carbon dioxide gas embolism. Reports have demonstrated that this event rate is low, particularly if the pneumoperitoneal pressure is maintained below 12 mmHg [40]. Studies have published and event rate of as low as at 0.5~1.5% [41]. There is a learning curve with gaining proficiency in the laparoscopic technique of liver resection with expert centers estimating the learning curve for laparoscopic liver resection at approximately 45~70 cases with senior partner proctoring [42]. Other limitations include the need for a skilled bedside assistant, and the diminished tactile sense when dealing with friable tissue such as steatotic liver parenchyma or thin venules within a cirrhotic liver can make the case challenging. And in the rare event when massive venous bleeding ensues, it can be difficult to control.

Cost is one major barrier to the wide adoption of the robotic approach. There is a significant initial capital investment in addition to maintenance fees and costs of staff training. However, one

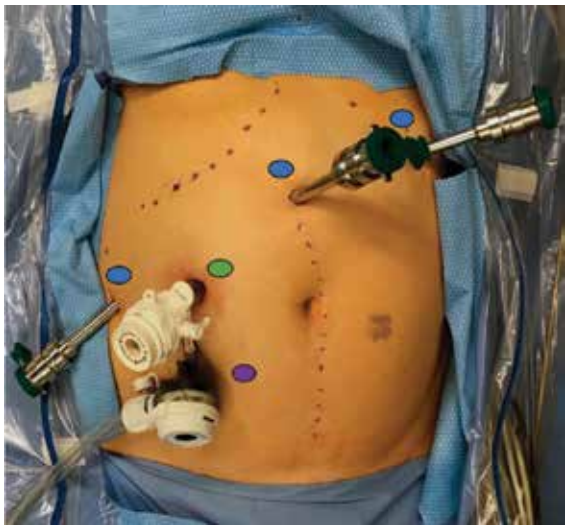

Figure 4. Image of port placement for a robot-assisted surgeries left lateral sectionectomy. Blue dots denote da Vinci 8-mm reusable cannulas (3). Green dot denotes 12-mm camera port. Purple dot denotes AirSeal® assistant port. Costal margin and midline marked in dotted pen.

Figure 5. Standard operating room set up for robotic-assisted liver surgery. Head of bed is on left side of image, anesthesia equipment and personnel on right side of image.

study demonstrated that while perioperative costs are higher with the robot, the overall total direct hospital costs are lower at least in part due to the decrease length of stay with robotic minimally invasive resection [43]. There are several generations of the robot with older generation units best suited for an operation in a single work field, with cumbersome redocking steps to perform multi-quadrant operations. The majority of studies indicate a longer operating time secondary to robot set up and draping. Technically speaking, the robot does not provide haptic feedback challenging the surgeon to "feel with their eyes" and occasionally resulting in excessive tissue damage in inexperienced hands. Further studies are needed to examine the comparative effectiveness of robotic versus laparoscopic minimally invasive hepatectomy.

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6. Postoperative complications

The main postoperative complications include postoperative hemorrhage, liver dysfunction, biliary leak and fluid collections. Postoperative hemorrhage is uncommon after liver resection if meticulous attention is given to confirmation of hemostasis at the conclusion of the case. Bleeding may occasionally occur from retroperitoneal structures, such as the adrenal gland, or diaphragmatic musculature. Argon beam coagulator and a variety of topical hemostatic applications are utilized to reduce liver surface related bleeding.

Post hepatectomy liver failure (PHLF) is a major postoperative complication with mortality of approximately 30%. The definition of post-hepatectomy liver is the impaired ability of the liver to maintain its synthetic, excretory and detoxifying functions, characterized by an increase in international normalized ratio and bilirubin on or after postoperative day 5 [44]. The most effective treatment of PHLF is liver transplantation but that is reserved for the most severe cases. Initial care is supportive and often includes mechanical ventilation, hemodynamic support and hemodialysis. Administration of colloid products and nutritional supplementation is also advocated.

The best way to treat post-hepatectomy liver failure is to prevent it. Preoperative weight loss, nutritional supplementation, careful preoperative selection and risk stratification are important to minimize the risk of PHLF [10]. Intra-operatively, minimizing blood loss and blood transfusion, close attention to hemostasis and minimizing skeletonization of the hepatoduodenal ligament will lower risk of PHLF. In the postoperative period, recognizing and aggressively treating postoperative hemorrhage, biliary obstructions or leaks and intra-abdominal infections will reduce the hepatic stress and likelihood of developing hepatic failure.

Postoperative fluid collections collect in the resected liver bed. These collections are varied in etiology but can include hematoma, seroma or biloma. They often to not result in symptoms, but occasionally they can cause pain or fullness requiring drainage. These collections also are at risk for infection and abscess formation. Biliary leakage from the raw surface of the resected liver can occur in up to 8% of patients after liver resection [45].

7. Emerging technologies

7.1. Near-infrared fluorescent imaging in hepatic surgery

New technologies continue to be developed to enhance minimally invasive liver surgery. One example is intra-operative near-infrared fluorescence (NIF) imaging. NIF imaging has become commonplace in many laparoscopic and robotic camera systems enabling the identification of various dyes, such as indocyanine green, injected preoperatively. Indocyanine green is a green dye that is preferentially metabolized by hepatocytes and excreted in the biliary tree. It lights up the biliary tree and has been utilized for robotic and laparoscopic assisted cholecystectomy. It has been more recently utilized to guide parenchymal dissection after vascular control by identifying perfused from poorly perfused hepatic parenchyma.

7.2. Intelligent imaging in robotic-assisted surgery

Future directions within the realm of robotic liver surgery include the application of preoperative planning with virtual reality (VR) models and real-time augmented reality (AR) intraoperative endoscopic overlays to aid with surgical navigation on *da Vinci* ® surgical systems. The current practice standard for operative planning involves preoperative cross-sectional imaging using contrast-enhanced, multiphase liver protocol computed tomography (CT) or magnetic resonance imaging (MRI) scans to evaluate the tumor's extent (size and number) and location with respect to critical structures including the major vasculature and biliary architecture. Surgeons rely on years of training to develop the ability to mentally reconstruct 2D images into a mental 3D model in order to preoperatively plan for a surgery while referencing the 2D images intraoperatively.

Computer-based three-dimensional (3D) reconstructions of liver tumors have been shown to increase accuracy of tumor localization and precision of operative planning for liver surgery [46]. While useful for operative planning, intraoperative review of 2D images on a traditional PACS system requires diversion of attention away from the operative field. Intraoperative ultrasound is routinely used for real-time localization of liver tumors and

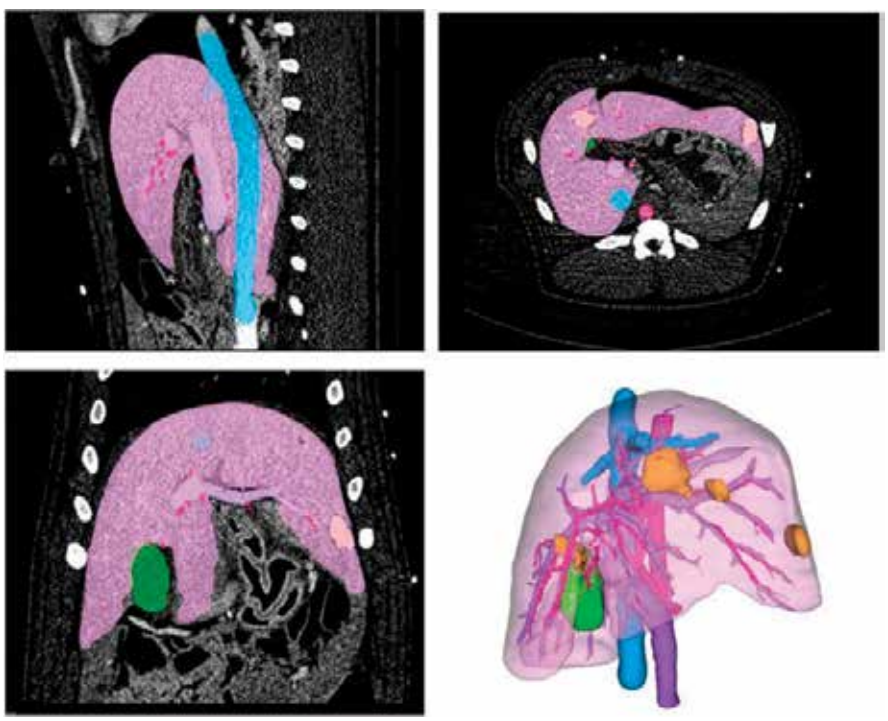

Figure 6. Virtual 3D model of the liver. Porcine experimental model with implanted radiopaque tumor within the liver parenchyma. Preoperatively, CT images were obtained of the porcine liver with 3D segmented reconstructions created from the DICOM images. The 3D reconstructions can be viewed for preoperative planning with intuitive Surgical's *da Vinci*® Surgical System.

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Figure 7. Real-time endoscopic overlay of 3D reconstruction over the surgical field on the *da Vinci* ® Xi Surgical System. The relationship between the tumor (light pink) and adjacent vasculature including the hepatic veins (light blue), hepatic arteries (red) and portal veins (blue) is present on the overlay. After initial registration, the overlay is mapped onto the patient-specific anatomy changing in real-time with camera movement.

identification of vessels and biliary structures. However, its use is limited in minimally invasive liver surgery due to the need for an additional port site and the need to interpret the 2D ultrasound images and mentally reconstruct the 3D anatomy being projected based on the orientation of the ultrasound probe. Preoperative planning with a VR model (**Figure 6**) and the application of AR endoscopic overlay (**Figure 7**) of patient-specific anatomy into the robotic surgical system could potentially improve surgical efficiency in real-time with intelligent surgical navigation.

AR may be developed to overlay accurate 3D reconstruction data onto the operative field itself, thereby eliminating the need to divert the attention from the operative field and to translate the 2D images into a 3D construct. These advancements with planning and guidance can potentially reduce the cognitive load burden on the surgeon. Augmented reality for spatial recognition has been shown to improve localization accuracy in an experimental model of uterine myomectomy [47],and our recent experience has shown promise and feasibility in an experimental porcine liver model (**Figures 1** and **2**). Next steps in the application of VR and AR to hepatobiliary surgery include overcoming technical obstacles of continuous co-registration to a mobile liver with tissue deformation while continuing to define the utility of the technology with patient education, tumor board evaluations, preoperative planning and intraoperative navigation.

8. Conclusion

Hepatocellular carcinoma (HCC) is a deadly disease that represents major challenges for patients and healthcare providers alike. Numerous therapeutic options exist for the treatment of HCC that are often used in combination for local and regional control. Surgical resection remains an important intervention that can be curative. Minimally invasive surgical technologies continue to improve increasing its safety and applicability for oncologic liver surgery.

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Conflict of interest

The authors have no conflict of interest to report.

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Chapter 5

Novel Techniques in the Surgical Management of Hepatocellular Carcinoma

Vasileia Ntomi, Anna Paspala and Dimitrios Schizas

Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy with cirrhosis preceding its development in most cases. Surgical resection remains the primary therapeutic option despite the recent emergence of locoregional therapies. Novel surgical techniques are being proposed to overcome the limitations of traditional anatomical open liver resection. Laparoscopic resection is a safe and effective alternative to open liver resection, especially for left lateral or peripheral segment tumors. It is associated with less postoperative morbidity, intraoperative blood loss, and medial hospital stay with no difference in oncological outcomes. Robotic-assisted liver resection overcomes the technically difficult resection of tumors located at the posterosuperior segments with similar outcomes to laparoscopic resection. Associated liver partition and portal vein ligation for staged hepatectomy (ALPPS) procedure allows resection in patients with HCC, and associated major vascular resection or small future liver remnant (FLR) with long-term results yet to be announced. For patients with small solitary tumors or poor liver function, non-anatomical liver resection is a feasible therapeutic option due to minimal postoperative morbidity and similar oncological results of anatomical resection.

Keywords: hepatocellular carcinoma, laparoscopic, robotic, ALPPS, novel

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer-related deaths worldwide [1]. Cirrhotic patients have the highest risk of developing HCC [2]. Numerous factors contribute to cirrhosis which precedes HCC development, including viral hepatitis, heavy drinking, and aflatoxin exposure. Hepatitis C epidemic in the Western world and Hepatitis B epidemic in China have attributed to the incidence of

HCC [3]. However, HCC has a dismal prognosis, mainly due to the early recurrence; about 40% of patients that have undergone hepatectomy develop recurrence within the first year after surgery [2].

Although liver transplantation is considered as the ideal treatment, hepatic resection remains the only curative method of therapy for HCC. Other methods of potentially curative therapy are radiofrequency ablation (RFA), microwave ablation (MWA), high power focused ultrasound ablation (HIFU), and transarterial chemoembolization (TACE) [4, 5].

Novel surgical techniques are being proposed to overcome the limitations of traditional anatomical open liver resection. Laparoscopic and robotic resection as well as nonanatomical resection and ALPPS procedure have emerged as new and effective ways of surgical therapy for HCC.

The aim of this chapter is to analyze the aforementioned novel surgical techniques in the management of HCC and present the results from the relevant studies.

2. Laparoscopic liver resection for HCC

Laparoscopic surgery has become widely accepted as a feasible alternative to traditional open surgery for many surgical indications. The first laparoscopic hepatectomy was performed in 1992, for a benign tumor by Gagner et al. [6], and the first laparoscopic resection for HCC was reported in 1995 [7].

The liver presents many and significant technical challenges for minimally invasive techniques. Its mobilization is difficult, the space is limited, its vascular and biliary anatomy is complex and the parenchyma is fragile, friable and often fibrotic or cirrhotic [8]. Nevertheless, numerous studies have already shown the feasibility and safety of wedge resections, single-segment resections, and left lateral sectionectomies [9, 10].

The first international consensus conference on laparoscopic liver resection (LLR) was held in Louisville in 2008. It was suggested that the best indications for laparoscopic excision were solitary lesions less than 5 cm, located in the anterior segments. Also, the resection should be far from the hepatic hilum and the vena cava [11]. The second international consensus was held in Morioka, Japan in 2014, stating that anatomical resection for HCC is standard of care procedure, but the laparoscopic version needs to be standardized to increase propagation [12].

There are many reasons why laparoscopic major hepatectomy has not been widely accepted and performed yet. There are technical difficulties related to liver mobilization, vascular control, inability for manual palpation, access to posterosuperior liver segments, and intraoperative hazards such as gas embolism, massive bleeding, and bile duct injury [13, 14].

The benefits of laparoscopic surgery, though, have long been proven. Early postoperative ambulation, decreased respiratory complications, minimization of blood loss, minimal abdominal trauma, and less postoperative pain are some of the accepted benefits of laparoscopic

surgery. For cirrhotic patients with HCC, the minimization of the surgical incision and the subsequent preservation of the abdominal wall circulation and lymphatic flow explains the decrease in postoperative liver failure and ascites formation [15].

The last decade, several meta-analyses of laparoscopic vs. open resection for HCC have been published [16–23]. These meta-analyses have analyzed and compared the results of many nonrandomized control trials and case-matched studies. Three categories of outcomes were used to compare the two operative techniques:

a. Operative outcomes, such as operative time, operative blood loss, and number of patients that needed transfusion.

b. Postoperative outcomes, such as morbidity, mortality, and hospital stay.

c. Oncologic results, such as pathologic resection margins, incidence of port-site recurrence, disease-free survival (DFS), and overall survival (OS).

Jiang et al. [16] reported the superiority of laparoscopic liver resection (LLR) concerning the reduced intraoperative blood loss and blood transfusion, the expansion of the pathologic resection margins, the increase of R0 resection, and the shorter length of hospital stay. Laparoscopic resection has similar OS, DFS, and recurrence rate as open liver resection (OLR).

Sotiropoulos et al. [17], in a recent meta-analysis of 44 studies, showed that laparoscopic resection is superior to open resection in terms of resection margin and R0 resection. It is possible that this difference in resection margin and R0 excision is due to the smaller size of tumors resected in the laparoscopic group. It was confirmed that the laparoscopic technique is strongly associated with less blood loss, fewer blood transfusions, less postoperative pain, faster recovery, and shorter hospital stay. Operative time and tumor recurrence were not statistically different between LLR and OLR as well as the long-term oncological results such as OS and DFS. These results confirm those of previous authors [15, 18, 21, 22]. Hand-assisted laparoscopic or laparoscopy-assisted resections (hybrid group) gain statistical advantage over the OLR group concerning the negative resection margin width and influence the results in favor of LLR. They, however, showed no difference as to the OS and 30-day mortality compared to the OLR group.

The main concerns about LLR are the inadequate tumor resection margins and the potential risk of port-site recurrence. Tumor recurrence is the main cause of death in patients with HCC. The adequate tumor-free margin is a prognostic indicator of HCC [23]. Due to the lack of tactile sensation in laparoscopic surgery, the tumor location is sometimes difficult to determine. Intraoperative ultrasonography is a useful tool for precise identification of lesions and its borders [24, 25]. Another concerning factor is the risk of tumor peritoneal dissemination and port-site metastases [26, 27]. Interestingly, there has not been any evidence so far of tumor peritoneal dissemination or port-site metastases [20, 22]. The use of a plastic bag to remove the specimen can help to prevent this complication.

Concern has also been raised about the safety of laparoscopic techniques in cirrhotic patients. A plethora of patients with HCC also suffer from cirrhosis. Portal hypertension is a major risk

factor for the development of postoperative decompensation [28, 29]. The benefits of LLR can be attributed to the preservation of the abdominal wall collateral circulation and the preservation of the round ligament which may contain significant collateral veins [18]. In a study by Tranchart et al., LLR had lower rates of liver decompensation, with the occurrence of postoperative liver failure and ascites ranging from 7 to 8% in LLR vs. 26–36% in OLR [30]. One study from Japan showed lower rates of morbidity, ascites formation, and shorter hospital stay following LLR with no difference in survival [31]. A recent meta-analysis presented intraoperative and postoperative outcomes of patients with known cirrhosis undergoing resection for HCC, comparing results for OLR and LLR [32]. This meta-analysis showed wider resection margins, reduced intraoperative blood loss and transfusion need, as well as reduced morbidity rates and shorter lengths of stay with the laparoscopic approach. Another study by Sotiropoulos et al. [33] mentioned the difference in results concerning cirrhotic patients that undergo LLR vs. OLR. The operative time was longer as anticipated, but the blood loss and morbidity had no statistical difference from the noncirrhotic group. The mortality rate was significantly lower in the cirrhotic subgroup when LLR was performed. Although patients with preserved liver function are the best candidates for LLR, cirrhotic patients benefit from LLR in terms of shorter hospital stay, complication rate, and long-term oncologic outcomes.

Tumor recurrence after primary HCC has been shown to be 30–70% at 5 years, limiting the overall survival of these patients [34, 35]. Numerous studies have been published reporting the results of repeat laparoscopic liver resection (RLLR) in patients with recurrent HCC [36–38]. A recent systematic review by Machairas et al. demonstrates RLLR as a safe and promising approach for the treatment of recurrent HCC, with significant benefits in terms of short-term outcomes with the oncologic adequacy not compromised [39].

The conversion rate has decreased from 5–15% [9, 40] to 4%, indicative of the surgeons' growing experience, with the most common causes being bleeding and failure to progress secondary to difficult exposure.

Overall, LLR can facilitate a safe and feasible approach to the surgical management of HCC. Major laparoscopic hepatectomy still remains a technically demanding procedure and should only be performed by highly experienced hepatobiliary surgeons with training in laparoscopic surgery. Longer follow-up periods are needed for more definite conclusions about the survival probability of the LLR vs. the OLR groups.

3. Robotic liver resection for HCC

Robotic liver resection (RLR) has been incorporated into clinical practice with increasing frequency since 2003 when the first report of a robotic liver resection was published by Giulianotti et al. [41].

Robotic technology was developed to overcome the technical difficulties of laparoscopic surgery; precision of movement, three-dimensional vision, magnification of the operative field, motion scaling, tremor filtering, and seven degrees of movement mimicking the human hand provide steady and careful dissection as well as prompt and precise endosuturing in case of

intraoperative bleeding. A major advantage of the robotic technology in liver surgery is the dissection of the hilum and the hepatocaval dissection in right hepatectomy [42] as well as the possibility of biliary reconstruction due to the microsuturing capacity of the robotic system [43].

All published liver resections were performed using the da Vinci Surgical System (Intuitive Surgical Inc., Sunnyvale, CA USA). The major disadvantage of robotic surgery is the high cost due to the longer operating time and the instruments required, in spite of the similar hospitalization costs [44]. The purchase and maintenance costs are significant, and that is the reason for the limited incorporation of the robotic system in many facilities.

A large series by Tsung et al. [45] compared RLR to LLR and with the exception of operative time, and they found no significant differences comparing operative and postoperative results of RLR and LLR. The R0 status did not change, and the oncologic margin was not compromised. It must be highlighted that using a minimally invasive technique, a greater percentage of minor and major hepatectomies was completed; 93% of RLRs were accomplished in a purely minimally invasive manner compared with 49.1% performed laparoscopically.

Chen et al. [46] compared RLR with OLR for HCC providing superior short-term outcomes for RLR (shorter length of stay and decreased need for patient-controlled analgesia) and similar long-term outcomes (DFS and OS) despite longer operative times for RLR. A substantial proportion of patients suffered from cirrhosis and half of patients underwent major hepatectomy. They reported a DFS in 1 year of 91.5% with the RLR, whereas DFS was 79.2%. Overall survival in 1 and 3 years did not differ between the two groups. The authors reported that the patients treated with RLR had significantly wider surgical margins compared with OLR. This matched comparison offers support for further RLR in patients with HCC, performed by experienced surgeons.

Another study by Lai et al. [47] presented the results of RLR vs. LLR for HCC. Robotic group had longer mean operating time (207.4 vs. 134.2 min). Both groups had similar blood loss (334.6 vs. 336 ml) and no difference in morbidity. Mortality rate was 0% in both groups. They reported a comparable 5-year DFS and 5-year OS between RLR and LLR (42 vs. 38% and 65 vs. 48%, respectively) in patients with HCC.

Salloum et al. [48] included 14 studies in their systematic review, with HCC comprising the majority of the malignant cases. Mortality was 0%, and overall morbidity ranged from 0 to 43.3%, results comparable to laparoscopy. The mean duration of LOS was similar in both techniques. There was no statistically significant difference between RLR and LLR concerning the surgical margins or R1 resections. No clear advantages of RLR over LLR were noted; therefore, it is difficult to establish the true indications for RLR. Nevertheless, RLR has the same advantages as LLR in terms of shorter LOS and postoperative return to normal activities. Also, it seems that the learning curve for RLR is shorter than that of LLR.

The most recent systematic review from Tsilimigras et al. [49] included 31 studies with HCC being the leading indication among malignancies, comparing RLR to LLR or OLR. Median operative time was 295.5 min, EBL was 224.5 ml, conversion rate was 5.9%, and complication rate was 17.6% in the RLR group. The complications were graded according to the Clavien-Dindo classification [50], with the most common complication being bile leak (2.9%). In minor

resections, the complication rate was 14.8% compared with the major resections, where the complication rate was 17%. Most of the studies show no benefit of RLR over LLR concerning safety and feasibility and multicenter, and randomized, prospective trials are needed to validate the exact indications and benefits of RLR.

Buchs et al. [51], in a systematic review of eight studies, compared RLR to LLR with the majority of the malignant cases being HCC (50.3%). There were minor and major hepatectomy procedures, and tumor size ranged from 8 to 120 mm. In the RLR group, there was no mortality, and the overall complication rate was 23.3% which fell to 19% when only postoperative complications were considered. A reduction of the conversion rate during major hepatectomy was reported as well. Overall, there was no clear outcome difference between RLR and LLR.

Ocuin et al. [52] included 14 major series in their review with the most common indication for resection being HCC. The estimated blood loss (EBL) ranged from 50 to 413 ml and transfusion rates from 0 to 44%. An overall conversion rate of 7% and an overall complication rate of 21% were reported. No perioperative mortality was associated with RLR. Length of stay (LOS) varied from 4 to 12 days. One study by Ji et al. showed a shorter LOS following RLR than OLR (10 vs. 7 days) [53]. Most series reported a high R0 resection rate with no port site recurrences. Recurrence rates following RLR were similar to those reported for LLR [9].

In conclusion, robotic liver resection is an acceptable alternative to open surgery with the robotic approach allowing an increased proportion of major hepatectomies to be performed in a minimally invasive manner [54]. These encouraging results should prompt the expansion of the robotic approach by highly specialized surgeons in experience centers worldwide.

4. Associating liver partition and portal vein ligation for liver surgery (ALPPS) for HCC

Surgical resection is the only potential curative treatment for hepatocellular carcinoma (HCC). In many cases, a major hepatectomy is required to achieve tumor-free surgical margins. However, the volume and functional reserve of the future liver remnant (FLR) are essential to avoid post-hepatectomy liver failure (PHLF), which is a crucial and important cause of morbidity and mortality after extensive liver resection [55]. In recent decades, some new strategies, such as portal vein embolization (PVE), portal vein ligation (PVL), and two-staged hepatectomy (TSH) have been developed to induce regeneration of FLR, minimizing the risk of PHLF and finally expanding the resectability criteria in HCC and generally in liver tumors [56]. Makuuchi et al. first introduced portal vein embolization into clinical practice in 1980s [57]. In 2015, a systematic review and meta-analysis from Pandanaboyana et al. compared PVL and PVE to assess the percentile increase of the

FLR, morbidity, mortality, and tumor progression [58]. This meta-analysis revealed that the difference in the mean percentile increase in the FLR between those two techniques was not statistically significant, with similar results in morbidity, mortality, and disease progression.

In 2000s, Adam et al. first described the two-staged hepatectomy for liver malignancies in which a single surgical procedure was not possible [59]. The primary reason for the failure of TSH is tumor progression between two stages or an insufficient hypertrophy in FLR after the first stage of the procedure (portal vein occlusion).

An innovative, accelerated two-staged technique utilizing PVL and in situ split (ISS) of hepatic parenchyma was first described in 2012 by Schnitzbauer et al. [60]. In the same year, De Santibanes et al. named this procedure as ALPPS procedure (associating liver partition and portal vein ligation for staged hepatectomy) [61]. In 2007, ALPPS was first performed by chance by German surgeon Dr. Schlitt [62, 63]. In an attempt to perform an extended right hepatectomy for a perihilar cholangiocarcinoma, he intraoperatively realized that FLR was inadequate. He resected the liver adjacent to the falciform ligament after performing a left hepaticojejunostomy. The right portal vein was also ligated for the purpose of left lobe hypertrophy. Out of curiosity, on postoperative day 8, he performed a computed tomography (CT) scan. To his surprise, the left lateral section had extensively grown in size. He successfully removed the diseased liver in a second operation.

ALPPS indications are an FLR < 30% in patients with a normal liver or an FLR < 40% in patients with a cholestatic, steatotic or fibrotic liver [64]. Therefore, ALPPS can be performed for marginally resectable or locally advanced tumors with an inadequate FLR. This technique constitutes a surgical strategy for colorectal liver metastases, hilar cholangiocarcinoma, and hepatocellular carcinoma [64]. On the other hand, contradictions for ALPPS procedure include unresectable liver metastases in the FLR, unresectable extrahepatic metastases, severe portal hypertension, high anesthetic risks, and a poor condition of the patient prior to this major operation [64]. Patients with cirrhotic liver are less capable for hypertrophy of FLR after portal vein obstruction (PVL or PVE) than patients with healthy liver parenchyma. Vennarecci et al. reported that ALPPS for HCC is safe even when performing a major hepatectomy in a cirrhotic liver. They also mentioned that ALPPS induces a significant increase in FLR between the first and the second stage of the procedure and after hepatectomy, either in healthy or cirrhotic patients [65].

It has been reported that postoperative morbidity and mortality after ALPPS are 16–64 and 12–23%, respectively, with the main cause of morbidity being bile leakage and sepsis and the main cause of mortality being PHLF [66, 67]. In the latest systematic review and meta-analysis by Zhou et al., 719 patients were included, and the aim was to compare the regeneration efficiency, safety, and complication rates of ALPPS and TSH. The degree of FLR regeneration in ALPPS was significantly higher than that in TSH, and the interval of the two stages in ALPPS was obviously shorter than that in TSH. Bile fistulas were much more common after ALPPS with the reason being the liver splitting that is mandatory during this procedure.

Although ALPPS had lower 1-year DFS rate, no significant difference in the 90-day mortality rate was discovered comparing the two techniques [66]. ALPPS was associated with a higher completion rate, a lower probability of tumor progression during the stage interval, and a lower insufficient regeneration rate; these findings are similar to those of previous studies [56, 67, 68].

Many variations of the ALPPS technique have been recently mentioned in the literature with the aim of improving safety and extending indications of hepatectomy. Modifications, such as avoiding liver mobilization and hepatoduodenal skeletonization, seem to prevent tumor spreading, adhesions, overall invasiveness, and parenchymal ischemia [69–73]. In addition, anterior approaches, portal vein embolization (PVE) as an alternative to ligation, partial liver splitting, tourniquet application or ablation procedures replacing parenchymal transection, and laparoscopic approaches represent fundamental modifications to the original ALPPS procedure that aim to improve safety [15]. The result of these modifications is the reduction of morbidity and mortality in this innovative surgical procedure. Furthermore, prospective controlled studies are needed to confirm which of these modifications should be considered as a reliable and safe alternative strategy to classical ALPPS.

5. Anatomical vs. nonanatomical resection for HCC

The incidence of HCC continues to increase due to the dissemination of hepatitis B and C virus infection. Hepatic resection is the gold standard treatment for HCC [74]. Nevertheless, postoperative recurrence of HCC, 3 and 5 years after hepatectomy is 50–60% and 70–90%, respectively [75, 76].

It is known that HCC invades mainly the intrahepatic vascular system and spreads along the portal and hepatic vein branches, producing intrahepatic metastases [77, 78].

Since Makuuchi et al. introduced the concept of anatomical resection (AR), the advantages of anatomic resection for HCC have been suggested in many studies [79]. On the other hand, limited nonanatomic resection (NR) with a minimal safety margin may be preferred for patients with impaired liver function [80]. Tanaka et al. showed that microscopic vascular invasion was more important than tumor size as a predictive factor for local recurrence [81].

Anatomical liver resection is a plausible option for patients with HCC, as HCC tends to cause intrahepatic metastasis through vascular invasion, and its advantages in improved OS or DFS for HCC patients have widely been reported [82].

In a systematic review of Cucchetti et al., AR seemed to yield improved 5-year OS and DFS compared to NR [83]. Zhou et al. [84] and Bigonzi et al. [85] presented significantly improved 5-year OS with AR.

Nonanatomic resection is recommended for patients with impaired liver function [86, 87]. The plausible reason is that NR can preserve as much functional liver as possible, with surgical curability and hepatic function equally important [87, 88]. The preservation of hepatic functional reserve allows effective treatment options in HCC recurrence, which may also improve the long-term prognosis [87, 89].

The superiority of anatomical resection (AR) over nonanatomic resection (NR) for hepatocellular carcinoma (HCC) remains controversial. Marubashi et al. reported no significant differences in OS, DFS or recurrence within 2 years after hepatectomy between the AR and NR groups [90]. Likewise, Tanaka et al. reported no outstanding difference in the recurrence rates and OS between AR and NR patient groups; it was also stated that survival rates after recurrence and median survival time after recurrence were higher in the NR group compared to the AR group for patients with a solitary HCC confined to 1 or 2 liver segments [91]. Chen et al. reported in their meta-analysis that AR contributed to better DFS, but did not improve OS [92]. Thus, the superiority of AR over NR is still controversial. Furthermore, Yamamoto et al. reported that AR is associated with more perioperative risks. The same study revealed significantly greater intraoperative blood loss and longer postoperative hospital stay for the AR group [82].

In 2010, Yamashita et al. [80] published a retrospective study of 321 patients with HCC. About 120 patients underwent limited nonanatomic resection (NR) for a single HCC < 5 cm. In noncirrhotic patients (n = 215), both 5-year OS and DFS rates in the AR group were considerably better than those in the NR group (87 vs. 76% and 63 vs. 35%, respectively). In cirrhotic patients (n = 106), both 5-year OS and DFS in the AR group were worse than those in the NR group (48 vs. 72% and 28 vs. 43%, respectively).

According to their results, the width of the resection margin did not influence postoperative recurrence, and major hepatic resections did not improve patients' survival. The main disadvantage of AR in comparison with NR is the limitation of a repeat resection, which would be the most effective treatment for recurrence, because of its disadvantageous effects on remnant liver function [93, 94].

In conclusion, there is a need for more, large, prospective, multicenter studies to confirm the data about any possible superiority of nonanatomic resection for HCC.

6. Conclusion

Hepatocellular carcinoma is a malignancy with an increasing incidence and a dismal prognosis. Patients are often referred to specialists in an advanced stage of the disease. Surgery is the primary treatment and novel surgical techniques are developed offering better perioperative and oncological results (**Table 1**). Nevertheless, prospective, randomized controlled studies have to be designed for the confirmation of such possible advantages of those new surgical techniques.

Technique	Advantages	Limitations	Reference
Laparoscopic resection	• Early ambulation • Decreased respiratory complications • Minimal abdominal trauma • Less postoperative pain • Decrease in PHLF • Reduced blood loss • Shorter length of hospital stay • Increase of R0 resection	• Technical difficulties • Vascular control • Difficult access to posterosuperior segments • Massive bleeding • No manual palpation • Longer operative time	[13–17, 23, 26, 27]
Robotic resection	• Precision of movement • 3-dimensional vision • 7 degrees of movement • Precise endosuturing • Dissection of the hilum • Biliary reconstruction • Shorter length of stay	• High cost • Longer operative time • Specialized surgeons	[42–44, 46, 54]
ALPPS	• Increase in FLR • Application to cirrhotic patients • Marginally resectable tumors • Locally advances tumors	• Severe portal hypertension • High risk patients • High rates of post-operative morbidity and mortality • Bile leakage • Sepsis	[64–67]
Nonanatomical resection	• Repeat resection • Impaired liver function • No difference in recurrence rates • Less intra-operative blood loss • Cirrhotic patients	• Worse 5-year OS and DFS • Width of resection margin • Microscopic vascular invasion	[80, 81, 83–85, 90, 91, 93, 94]

Table 1. Advantages and limitations of novel surgical techniques for hepatocellular carcinoma management.

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Section 4

Metabolic Impact on Liver Cancer and Alternative Medicine

Chapter 6

Metabolic Risk Factors in Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver and it is one of the leading causes of cancer-related deaths worldwide. The global burden of hepatocellular carcinoma is growing nowadays. Most cases of hepatocellular carcinoma develop in the background of chronic hepatitis C and B and liver cirrhosis-well-known risk factor. But despite the reducing incidence of chronic hepatitis infections, an increase in the incidence of hepatocellular carcinoma was observed in the last decades. This could be explained by the increasing prevalence of obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), which are becoming important risk factors in hepatocellular carcinoma. Regular surveillance, as performed for patients with viral hepatitis, is required for patients with metabolic risk factors.

Keywords: hepatocellular carcinoma, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis

1. Introduction

Hepatocellular carcinoma (HCC) is one of the dominant histopathological types of liver cancer, accounting for almost 90% of primary liver cancers worldwide, it is the sixth most common cancer and it is the third cause of cancer-related deaths worldwide [1]. Despite the decreasing incidence of HCC related to viral hepatitis, an increase in the incidence of HCC was observed especially in Europe and America [2]. The global burden of hepatocellular carcinoma in 2012 was of 14 million cases and it is predicted to grow to 22 million over the next two decades.

The most common and known risk factor for HCC are viral infection, virus B or C, toxic factors - alcohol and aflatoxin and immune diseases like primary biliary cirrhosis. There is an increasing number of HCC developed on liver metabolic diseases, including NAFLD and NASH, based on epidemiological evidence that shows a relationship of these diseases with an incident of HCC, regardless of the common known risk factors like alcohol consumption or chronic viral hepatitis.

It is not surprising the growing interest in the last few years on the mechanisms underlying the transition from liver metabolic disorders to HCC that is involving these new metabolic risk factors that include inflammation, insulin resistance, lipid and bile acids metabolism and the gut microbiota. A better understanding of the impact of these factors on the liver microenvironment may have potential benefit on the management of liver disease [3].

Metabolic syndrome has been associated with an increased risk of HCC and each component of this syndrome may increase cancer risk and also a synergic effect has been described [4, 5]. Overweight and obesity are well recognized independent risk factors for HCC, visceral adiposity showing stronger association with HCC risk than general body weight [6, 7]. Studies demonstrated that obesity may also influence HCC prognosis, Body Mass Index (BMI) seems to be a predictor of microvascular invasion and poor prognosis, while visceral adiposity is associated with HCC recurrence after treatment [8, 9]. Type 2 diabetes mellitus has been recognized in various studies as an important independent risk factor for HCC regardless of alcohol consumption [10]. Hyperlipidemia and hypertension are two additional components of metabolic syndrome that have been studied as risk factors for HCC, and hypertension seems to be related to a higher risk of HCC, whereas the relation between HCC and hyperlipidemia remains controversial [4, 5]. Also, synergism between the new risk factors and traditional risk factors has to be considered, for example, a strong synergic effect of alcohol abuse and type 2 diabetes mellitus has been described, also diabetes and obesity have been reported to enhance the risk of HCC in patients with chronic hepatitis [11, 12].

Non alcoholic fatty liver disease (NAFLD) is one of the most common cause of chronic liver disease and include a large spectrum of chronic liver disorders ranging from simple hepatic steatosis with no evidence of hepatocellular injury to non-alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis and liver failure and currently, all guidelines agree that NAFLD is associated with the increasing percentage of obesity, type 2 diabetes mellitus, hypertension and dyslipidemia in our population [13, 14]. Several studies have demonstrated a strong association between NAFLD and each components of metabolic syndrome and there is enough evidence to define NAFLD itself as a liver component of metabolic syndrome [15–19, 46].

The rising incidence of NAFLD/NASH worldwide led to an important rise in HCC incidence related to these chronic liver diseases [3]. Many studies have demonstrated over the years that NAFLD can lead to hepatic fibrosis and cirrhosis, increasing therefore the risk for developing HCC [20, 21]. Among these patients with Non alcoholic fatty liver disease or non-alcoholic steatohepatitis, studies show that the third cause of death is liver disease, and HCC represents the main cause of death in these patients [20–23]. The incidence rate for developing HCC in patients with NASH related hepatic cirrhosis is up to 27% in retrospective studies [24]. Increased incidence of HCC was also been reported in patients with NAFLD in the absence of hepatic cirrhosis, and several risk factors for HCC development have been identified [6, 25–29].

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2. Natural history: Progression from hepatic steatosis to HCC

The term nonalcoholic fatty liver refers to a variety of liver disease that ranges from simple isolated hepatic steatosis, to non-alcoholic steatohepatitis with or without cirrhosis, and progression to HCC.

Although NAFLD diagnosis can be made by imaging (ultrasound or magnetic resonance), biopsy still remain the gold standard for diagnosis. Histology generally displays the accumulation of triglycerides in hepatocytes, usually in mixed macrovesicular or microvesicular droplets, in the absence of alcohol abuse, steatogenic medication or hereditary disorders [30].

The prevalence of NASH is difficult to determine because biopsy is required, with specific criteria such as steatosis, hepatocellular injury, mainly in the form of ballooning, and lobular inflammation, and once cirrhosis is present, NASH may be difficult to evaluate because often the fatty deposition disappear. Liver fibrosis may be present in non-cirrhotic NASH, initially in perisinusoidal acinar zone 3 [31]. Because of the need of histopathologic confirmation, NASH is most likely underdiagnosed and it may be misclassified as cryptogenic cirrhosis, which shares the same risk factors including diabetes and obesity [32, 33, 46]. Therefore, to estimate correctly the prevalence of NASH, a novel NASH category including obese patients with cryptogenic cirrhosis or with unknown HCC etiology has been proposed.

The prevalence of NAFLD and NASH is variable and it is depended on the method of diagnostic used to confirm the disease, and it is usually underreported because of the asymptomatic nature and can be underestimated and poorly treated.

NAFLD is present in more than 25% of adult population and about 10 to 20% of NAFLD patients may progress to NASH, which may progress to cirrhosis in 20–45% of cases, and cirrhosis is a well-known risk factor for HCC, and approximately 7% of patients with NASH-related cirrhosis may progress to HCC within 6 years [31].

Patients with nonalcoholic steatohepatitis are more susceptible to develop progressive advanced liver disease when compared to benign course of simple hepatic steatosis. In a study that included 420 patients with NAFLD/NASH, it was demonstrated a higher mortality in these patients when compared to the general population and also liver-related deaths occurred in 13% compared to 1% in general population, and 3% of patients with NAFLD developed hepatic cirrhosis. [34] Another study showed increased rates of hepatic cirrhosis in patients with non-alcoholic steatohepatitis (25%) compared to patients with fatty liver without non-alcoholic steatohepatitis (3%), and also showed an increased risk of liver disease related death in these patients (11% *vs* 2% in patients with fatty liver without NASH) [35].

Patients with compensated liver cirrhosis related to non-alcoholic steatohepatitis present with better survival outcomes compared to patients with HCV related cirrhosis, but in the presence of uncompensated liver cirrhosis poor prognosis was observed in both populations [36, 37], and currently, both, the American and European Associations for the Study of Liver Diseases, recommend screening for HCC in all patients with non-alcoholic steatohepatitis related cirrhosis [38].

Evidence from studies suggests that an important proportion of patients with NAFLD-associated HCC, do not have histologic evidence of liver cirrhosis. In one study from 1168

patients that underwent hepatic surgery for HCC, 6 out of 8 patients with NASH-related HCC did not had any histopathological evidence of liver cirrhosis and also the study suggested that the presence of hepatic cirrhosis in NASH-related HCC patients is lower compared to HCV-related HCC [29].

In another study that analyzed 128 patients with HCC recruited over a period of 12 years, it was reported that a significant number of patients with NASH developed HCC in the absence of fibrosis when compared to HCC of other etiology [39]. To explain this phenomenon in non-cirrhotic NAFLD patients, one proposed hypothesis is the malignant transformation of liver cell adenoma, and there are some published reports that have suggested that in the presence of metabolic syndrome features, liver cell adenoma may incur a malignant transformation [40, 41].

In the last years, many studies tried to establish the relationship between NAFLD and NASH, cryptogenic cirrhosis and HCC. The true prevalence of NASH and NASH-related HCC is probably underestimated due to the asymptomatic nature of the disease, and in up to 29% of HCC cases, the underlying etiology of liver disease remains unknown or are considered as cryptogenic cirrhosis [40]. Histopathological features that are suggestive for non-alcoholic steatohepatitis are more frequently observed in patients with HCC of unknown etiology than in patients with HCC related to chronic viral hepatitis or alcoholic etiology [32]. Even if the true prevalence of NAFLD/NASH-related HCC is not yet well defined, the increasing incidence of obesity and diabetes, suggests that the incidence NAFLD/NASH-related HCC will continue to grow in the next years, and there are already numerous studies that are investigated the relation between these diseases [46].

3. Metabolic risk factors, NAFLD and HCC

It is established that HCC and NAFLD share many risk factors and the development of HCC in NAFLD/NASH patients is probably multifactorial and involves low grade chronic systemic inflammatory response, excessive fat accumulation and insulin resistance [40, 46].

3.1. Obesity

There is more evidence that overweight and obesity and metabolic syndrome have reached a epidemic proportion over the last decades, and there are evident data that show that 80% of NAFLD patients are overweight or obese [42]. According to the World Health Organization, in 2008, more than 35% of adults worldwide are overweight, and of these, 13% are obese [29] and if overweight and obesity rates continue at their current ascending trend, it is estimated that more than 3.3 billion adults will become overweight or obese by year 2030 [43].

Overweight and obesity are leading risk factors for overall mortality, accounting for more than 3.4 million adult deaths every year, and are considered risk factors for 44% of the diabetes, 23% of the ischemic heart disease, and between 7 and 40% of certain cancer [42].

Body Mass Index is the most commonly used index in epidemiologic studies, but body fat topography, and especially central obesity, seems to be more important in pathophysiologic mechanisms that connect obesity to cancer. Central obesity, is the key feature in most metabolic syndrome definitions, and has also been directly correlated with insulin resistance [44, 46].

Obesity have been associated with disproportion between visceral and subcutaneous adipose tissue and with chronic inflammatory state due to adipokine imbalance that is defined as increased levels of leptin and decreased levels of adiponectin. Furthermore, obesity has been associated with other risk factors including insulin resistance, increased hepatic lipid storage and alteration of intestinal flora [46].

Adipokine imbalance as mentioned before occurs with simultaneous increased leptin and decreased adiponectin levels resulting in a pro-inflammatory and pro-oncogenic state. Both leptin and adiponectin are hallmarks of obesity, and have been extensively studied and both have been related to NAFLD and progression to liver cancer.

Leptin is secreted by adipose tissue and acts as a hormone and it is involved in the process of satiety. High levels of leptin and resistance to its action are observed in obese persons. Leptin has been demonstrated to be implicated in NAFLD progression, liver fibrosis, NASH and eventually in the carcinogenesis process of HCC through multiple molecular mechanisms. And these mechanisms are the activation of JAK2/STAT3, PI-3 K/Akt, ERK pathways and the inhibition of the TGFβ1-induced apoptotic pathway [24]. For example, the activation of Akt pathway was observed in about 40% of HCC patients. Leptin's role is to have growth factor-like activities on hepatic cells and HCC cells, and also have proinflammatory, profibrogenic and proangiogenic role on liver microenvironment and also it is implicated in the process of cell growth, angiogenesis and metastasis [31].

Adiponectin is the most abundant hormone of adipose tissue and has well known metabolic functions, having anti-inflammatory, antifibrotic, antiangiogenic, and antiproliferative activities on the liver microenvironment. Adiponectin exerts antifibrotic effects on hepatic cells through activation of the signaling AMPK axis and inhibition of TGFβ-mediated profibrogenic gene expression, and in addition, adiponectin may also induce apoptosis of hepatic cells. The anti-inflammatory activity of adiponectin is mostly related to inhibition of NFkB signaling axis [31]. A direct effect of adiponectin on HCC cells has also been described, induces apoptosis and inhibits HCC cell proliferation and migration. In addition, adiponectin prevents HCC development by activation of the AMPK signaling pathway and consequent modulation of mTOR and JNK/caspase 3 axis, resulting in growth cell inhibition and enhanced apoptosis [4]. A number of observations support the reduced adiponectin levels observed in obese patients and were associated to increased incidence of hepatic steatosis, fibrosis and accelerated progression to HCC [45].

3.2. Insulin resistance

Insulin resistance it is another important component of the metabolic syndrome, and along with obesity, is involved in the chronic inflammatory state directly linked to NAFLD. Insulin

resistance is also related to oxidative stress, which has the most important role in carcinogenesis in the presence of NAFLD and NASH.

Epidemiologic studies show that diabetes is associated with an increased risk of developing HCC compared with non-diabetics patients, regardless of other HCC risk factors and also seems to be independent of obesity [47]. In a large study conducted on patients with and without diabetes, with a follow-up period of 10–15 years, NAFLD incidence was significantly higher among patients with diabetes and a significantly higher incidence of HCC among patients with diabetes was observed [7]. Meta-analysis published over the years, revealed a 2 to 3-fold greater risk of HCC in patients with diabetes compared with non-diabetic patients, and this significant association was reported independent of alcohol abuse or chronic viral hepatitis in studies that examined these factors [48, 49].

Epidemiologic data demonstrate that both obesity and type 2 diabetes mellitus have increases the risk for HCC, and NAFLD, which is present in up to 90% of obese persons and up to 70% of type 2 diabetes mellitus patients [24], appears to play an important role in HCC development. NAFLD is nowadays considered the most common risk factor for HCC, followed by type 2 diabetes and it is exceeding the incidence of chronic viral infections and alcoholic liver disease [48, 50]. These can be explained by effective measures to reduce HCV infection incidence, which was the major cause of HCC in the United States and in other developed countries, and also can be explained by the increasing prevalence of NAFLD in these areas [51].

The strong relationship between visceral obesity and insulin resistance (IR) is well known, but insulin resistance is not related only to adipose tissue, in fact, liver accumulation of fatty acid metabolites can induce hepatic insulin resistance. One of the main fatty acid metabolite involved in hepatic insulin resistance is diacylglycerol (DAG) and it has been proposed as a predictor for hepatic insulin resistance [52]. The consequent hyperinsulinemia downregulates s expression of IRS2 in the hepatic cells increasing hepatic insulin resistance and in addition, insulin stimulates lipogenesis through activation of SREBP-1c, inducing, in a vicious circle with further fat accumulation and insulin resistance [31]. The liver microenvironment may induce insulin resistance also in other tissues, in fact, an increase in liver fat content may be considered a very strong predictor of insulin resistance in skeletal muscles, hepatic and adipose tissue, regardless of adiposity. In conclusion, liver fat content may predict the development of metabolic syndrome or diabetes, and the underlying mechanism may be the altered gene expression and protein synthesis and secretion also observed in NAFLD [53]. It is known that hyperinsulinemia occurs as a response to insulin resistance, and that is considered a risk factor for liver fibrosis and HCC development by activation of hepatic stellate cells, by dysregulation in the proliferation-apoptosis balance in hepatic cells, and by stimulation of angiogenesis. The most studied mechanism involved in NAFLD-related HCC is the IGF signaling axis that has a growth factor-like activity on hepatic cells and also a pro-angiogenic activity on the hepatic vascular system. Dysregulation of the IGF signaling axis has an important role in hepatic carcinogenesis and it is represented by the low levels of IGF1 in serum and overexpression of IGF-II. Insulin receptors (IRS) bind to insulin or IGF and share the same pro-oncogenic pathways with IGF1 receptor (IGF1R), including the activation of P13K/Akt and MAPK [54, 55].

3.3. Lipotoxicity

Increased lipid accumulation in the liver arises from lipolysis within peripheral adipose tissue, dietary sources and de novo hepatic lipogenesis, and this increased lipid accumulation causes hepatic lipotoxicity resulting in the excessive production of saturated and monounsaturated free fatty acids (FFAs) [40, 46, 56]. These FFAs undergo β-oxidation leading to formation of reactive oxygen species that will further induce mitochondrial damage, endothelial reticulum stress, and gene transcription promoting inflammatory cell signaling pathways.

As a result of the hepatic insulin resistance an increase in the liver of free fatty acids (FFAs) is observed, mainly due to dysregulation of the lipolysis and lipogenesis balance, resulting lipotoxicity that will determine chronic damage to hepatic tissue [51]. But, lipotoxicity is not due only as consequence of the excessive accumulation of FFAs in the liver, and the modification of lipid composition is another contributor to lipotoxicity, and recent studies are aimed at searching for specific metabolic changes as potential signatures of development of HCC in patients with NAFLD [57]. For example, some studies show that, during natural history of progression from normal liver to NAFLD or NASH, the ratio of polyunsaturated fatty acids (PUFAs) is increased in NASH, and phosphatidylcholine (PC) levels are reduced in both NAFLD and NASH, and based on these observations, it has been suggested that the LPA signaling axis may be one of the mechanism that is connecting hepatic steatosis to HCC [58–61].

3.4. Microbiota: Intestinal flora dysregulation

The basis for the ongoing interest on the role of gut microbiota in progression of NAFLD was the observation of fatal NASH that occurred in patients undergoing jejunoileal bypass in bariatric surgery and the reversal after metronidazol therapy [62]. There are several evidences that demonstrate a high prevalence of small intestinal bacterial overgrowth in patients with NAFLD/NASH and that also demonstrate the role of microbiota modifications in the development of NAFLD and NASH [45]. Specific microflora changes may play an important role in progression of hepatic steatosis, especially in obese patients. In patients with NAFLD and NASH was observed a difference in microbiota composition compared with healthy population [63, 64]. The mechanisms implicated in the progression of gut microbiota-related NAFLD and NASH and HCC are: alteration of intestine permeability, persistent activation of innate immune system with consequent chronic inflammation, changes in bile acid metabolism [65].

Patients with NAFLD or NASH show increased levels of lipopolysaccharides (LPS), a known innate immune system activator, on serum confirming the inflammatory state associated with this conditions and alteration in gut permeability with disruption of intercellular tight junctions observed in patients with NAFLD can contribute directly to lipopolysaccharides action to the liver [65, 66].

All these findings were confirmed in human study wherein increased LPS-binding protein (LBP) levels were observed in obese patients with NAFLD and even more in obese patients with NASH, correlating with liver TNFα increased expression [67].

All these mechanisms show how changes in the microbiota, in combination with loss of innate immune sensors, may induce metabolic liver disorders.

Gut microbiota also influences bile acid metabolisms mainly through the stimulation of the bile-acid-activated nuclear receptor and also by interacting with farnesoid X receptor (FXR) which induce excretion of bile acids from the liver and production of antimicrobial peptides [65].

4. Conclusions

Although significant progress has been made in NAFLD/NASH related HCC, many issues still remain to be resolved. With the prevalence of HCV declining in the last years, the incidence of NAFLD/NASH is expected to account for a greater proportion of HCC incidence in the near future due to the growing epidemic of obesity, diabetes and metabolic syndrome, known as independent metabolic risk factors for development of HCC. The annual incidence rate of HCC developed in patients with NASH-related cirrhosis is not yet clearly established and recent evidence show that a significant number of patients with NAFLD or NASH progress to HCC in the absence of hepatic cirrhosis. NAFLD/NASH-related cirrhotic patients receive significantly less surveillance for HCC than those with HCV-related cirrhosis, in contrast to epidemiological data and this represents an important public health problem. Also dysbiosis play an important role in progression of liver disease via changes in bile acids metabolism and dysregulation of intestinal barrier.

In conclusion, metabolic syndrome comprising of obesity, type 2 diabetes, dyslipidemia, hypertension, is related with an increased risk for development of HCC. NAFLD considered the liver manifestation of metabolic syndrome is an important factor implicated in progression to HCC. Also alteration in gut microbiota seems to be connected with HCC occurrence but many questions still remain to be answered.

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Conflict of interest

The authors declare no conflict of interest regarding this review.

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Chapter 7

What Chinese Medicine Can Do for Liver Cancer?

Feiyu Chen, Ning Wang and Yibin Feng

Additional information is available at the end of the chapter

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Abstract

Liver cancer is an international problem, especially in Asian countries. It is because that most liver cancers are already late stage when they are diagnosed, and also most liver cancers have various previous chronic liver diseases induced by alcoholic, virus, and steatosis, etc. In recent years, laboratory and clinical studies focusing on liver cancer by Chinese medicine has been extensively studied. What Chinese medicine treatment formalities can be used in liver cancer? How Chinese medicine can be employed in treatment of liver cancer? What Chinese medicine can contribute to liver cancer? To answer these questions in this chapter, we will review and discuss treatment of liver cancer from Chinese medicine's perspective with scientific evidences as following three parts: (1) Chinese medicine as the source of discovering new treatment for liver cancer, (2) Chinese medicine as a complementary treatment of liver cancer, and (3) to discuss future research and application of Chinese medicine in liver cancer treatment.

Keywords: Chinese medicine, liver cancer, source of drug discovery, complementary medicine, clinical application

1. Introduction

Liver cancer is one of the most common malignancies with high morbidity and mortality all over the world. Despite the number of new cases of liver cancer appears to be plateauing, large population size of liver cancer patients, especially in China, still greatly contributes to the global cancer deaths [1]. Hepatocellular carcinoma (HCC) is the most commonly observed histological subgroup of primary liver cancer, accounting for 70–90% of the cases. With a global status quo that 746,000 deaths only in 2012 and 10.1 new cases diagnosed within every 10,000 people, HCC ranks the sixth lethal malignancy and the third leading cause of cancer-related deaths [2].

Over the past decades, the clinical approaches to treat liver cancer have considerably evolved. Patients can benefit from partial hepatectomy, radiotherapy, systemic or local chemotherapy, liver transplantation, and radiofrequency ablative surgery. Nevertheless, numerous adverse events and dismal outcomes still seriously affect the life quality of patients. On the background of shortcomings, developing improved preventive and therapeutic strategy is urgently necessary.

Considering its low toxicity and high activity, Chinese medicine has been deemed as one of the prominent complementary and alternative approaches in tumor therapy. As unique biomedical and pharmaceutical resources, Chinese medicine owns the ability of providing better treatment for liver cancer, either alone or in integrative way [3]. According to Hong Kong Liver Cancer staging system in a population-based investigation, for patients with Va/Vb (tumor status being early, intermediate or locally advanced), the most frequent treatment was Chinese medicine [4]. Another cohort study in Taiwan reported that Chinese medicine users exhibited significant lower risk to suffer HCC, which supported the application of Chinese medicine into the clinical practice of liver cancer treatment [5]. A recent meta-analysis showed that add-on therapy with Chinese medicine regimens in HCC could reduce side effects, activate tumor responses, and improve overall survival. Moreover, cancer subjects were reported to be more inclined to integrating Chinese medicine regimens with conventional therapies rather than conventional treatment only [6]. In this regard, Chinese medicine has been considered as a potential curative choice of method for controlling the proliferation of liver cancer, and thus improving the quality of life and prolonging overall survival of the patients.

Historically, the medical foundation of Chinese medicine can be traced back to 5000 years ago. With contributions and dedications of Chinese medical people in modern and old times, Chinese medicine has been gradually evolved and accepted by the mainstream society. In particular, accompanying the tide of Chinese immigration and cultural communication, Chinese medicine has been approved worldwide and employed in clinical practice in at least 183 countries [7]. Even though many regions have the regulations imposing restrictions to ensure that Chinese medicine is beneficial to liver cancer patients instead of being harmful to public health, the evidence-based guideline has not been covered every field [8, 9].

However, due to its effective curative outcomes in real life, the usage of Chinese medicine in various forms of single compounds, extracted fractions, and composite formulae has attracted a great deal of attentions over the past few decades. Chinese medicine may be capable of retarding liver cancer progression with its multitargets and coordinated intervention actions, either in combination with conventional therapies or radiation alone. Here, we retrospectively reviewed and analyzed the functional roles of Chinese medicine in the treatment of liver cancer.

2. Chinese medicine as the source of discovering new treatment for liver cancer

As mentioned above, currently there are various therapies for liver neoplasm. However, the overall survival rate of patients still remains unsatisfactory on account of high invasiveness

and metastasis, chemotherapeutic resistance, and so on. Chinese medicine, in various forms including composite formulae, extracted fractions, monomers, and their derivatives, has been pursued as ideal and novel sources for therapeutic agent development for cancer.

2.1. Single compounds from Chinese medicine for the treatment of liver cancer

Berberine is a natural product in many Chinese medicinal herbs, especially *Coptidis rhizoma*, which has been extensively studied and reported to show the antitumor action mostly by modulation of a number of different signal transductions. Currently, scholars have explored the antitumor action of berberine in liver cancer by various different strategies. For instance, in our laboratory, we found that berberine-induced cell death and tumor growth inhibition in xenograft model were demonstrated and mechanism was revealed that miR-23a might play a mediated role in berberine-suppressing HCC growth [10]. Also, cyclin D1 overexpression is mainly responsible for tumor expansion, metastasis as well as angiogenesis. Berberine was found to repress the expression of cyclin D1 via proteasomal degradation in HCC [11]. In addition, our group identified that berberine exerted antimigratory and anti-invasive abilities in HCC cells involving the upregulation of PAI-1 and downregulation of uPA [12]. On the other hand, our group described for the first time that berberine could trigger autophagic cell death, in which the compound was shown to activate Beclin-1 and suppress mTOR [13]. Actually, lung metastases in liver cancer are also a serious problem for patients, and we identified that the anti-invasive and antiproliferative actions of berberine in liver cancer was at least in part involved in the downregulation of Id-1, revealing a new anti-invasive mechanism [14]. Hence, berberine is predicted as a new and potent natural molecule targeting liver cancer.

Flavonoids commonly exist in Chinese medicine and could be isolated from many different kinds of herbal medicine. In recent years, the precise molecular mechanism underlying the obvious antiliver tumor effect of flavonoids has been studied. For example, hydroxysafflor yellow A (HSYA), a kind of flavonoid extracted from *Carthamus tinctorius* L. owns the ability of antitumor. It was demonstrated that HSYA could result in angiogenesis inhibition of HCC by blocking signaling pathways of ERK/MAPK and NF-κB in comparison with negative control group. More interestingly, spleen and thymus indexes have been demonstrated to be improved, suggesting improvement on the immune system by HSYA [15]. Oroxin B (OB) is one of the flavonoids isolated from *Oroxylum indicum* (L.) Vent. Li et al. investigated the antitumor effects of OB on HCC cell line SMMC-772 and studied the underlying mechanisms by which OB markedly inhibited expansion and induced apoptosis of the HCC cells. The antitumor activity of OB probably involved the inhibition of COX-2/VEGF and PTEN/PI3K/AKT signaling pathways, providing evidence for OB being used as a new therapeutic agent for liver cancer [16]. Another flavonoid, namely luteolin, showed antineoplastic activity in a number of cancer cells. In SMMC-7721 HCC cells, luteolin induced apoptosis partially via modulation of autophagy, indicating luteolin serving as a regulator of autophagy in treating liver cancer [17].

Brucein D (BD) is an active constituent derived from *Brucea javanica* fruit, which has been employed as an antitumor recipe in Chinese medical practice. It was revealed that BD exerted observable apoptotic induction in HCC in vitro and in vivo, which was attributed to the reduced expression of miR-95 [18].

Matrine, a chemical component came from the roots of sophora species, mainly *Sophora flavescens* Ait (SF), has been used clinically to treat diseases such as liver fibrosis. The hepato-specific *miR-122a* has been found decreased in HCC cell lines [19]. Zhou et al. reported that in HepG2 cells, matrine could cause cell arrest alteration as well as apoptosis induction with recovering expression of miR-122a [20]. Actually, matrine is the prominent bioactive compound in one adjuvant treatment of liver cancer, namely Fufang Kushen injection, which was approved by Chinese FDA in 1995. Matrine has been deemed as the favorable lead source for drug discovery owing to its changeable structure and stable safety profile. Researchers designed and synthesized a group of matrine derivatives, which improved the antitumor activities of matrine in several human cancer cell lines. Among four tested cell lines, HCC cell line Bel-7402 responded more sensitively to compounds than the other three cell lines. Matrine and its derivatives induced G1 cell cycle blockage as well as migration inhibition in HCC cells [21]. Another matrine derivative named WM622 showed remarkable inhibitory effect on HCC both in vivo and in vitro. Further study showed the apoptotic induction, cell cycle blocking in G0/G1 phase and the inhibition of PI3K/AKT signaling were involved in the antiliver cancer effect of WM622 [22].

Longikaurin A (LK-A) is a naturally occurring compound of ent-kaurane obtained from *I. aternifolius*. Researchers explored LK-A administration in liver of tumor-bearing mice models and discovered that LK-A could induce cell cycle arrest at G2/M phase with downregulation of Skp2 and subsequently resulted in induction of ROS/JNK/c-Jun apoptotic pathway in HCC cells [23].

The antitumor of two known pennogenyl saponins, which are derived from *R. paridis axialis*, was investigated in orthotopic nude-mouse model. The data indicated that these two monomers dose dependently suppressed the HCC progression through activating both caspase-independent and caspase-dependent apoptotic pathways. Furthermore, possible mechanism probably involved the modulation of mitogen-related protein kinase pathway as well as the suppression of PI3K/Akt signaling [24].

Isoquercitrin was found to strongly repress liver tumor cells via retarding the G1 phase cell cycle and promoting cancer cells apoptosis. In nude mice, the proliferation of transplanted tumors was suppressed after treatment with isoquercitrin. Further study showed that the underlying mechanism might be closely involved in the MAPK and PKC signaling pathways [25].

Zhang et al. investigated the effect of astragaloside IV (AS-IV) and curcumin on tumor expansion and angiogenesis in nude mice bearing xenografts of HCC. Combining AS-IV and curcumin revealed significant synergistic repressive efficacy against both angiogenic and thrombosis-related factors, which might be mediated by downregulation of *miR-221* as well as upregulation of *miR-122*. This current study indicated future clinical potential of combination therapy with AS-IV and curcumin for treatment of liver cancer [26].

Ursolic acid (UA), a naturally occurring pentacyclic triterpenoid carboxylic acid found among Chinese herbal medicine, has been reported to be a potent component for cancer prevention, including liver cancer. Yie et al. explored the probable mechanisms underlying the antiliver cancer action of UA. Taken together, the results demonstrated that UA inhibited proliferation

and induced apoptosis of HCC cells via AMPKα-mediated suppression of Sp1, followed by suppressing DNMT1 expression. The investigation revealed a potential novel mechanism by which UA controlled proliferation of HCC cells, suggesting the critical effect of DNMT1 in HCC chemoprevention and treatment [27].

Bilobol is a Chinese medical ingredient. Xu et al. identified that bilobol administration could suppress expansion of HepG2 cells, which pretreated with lipopolysaccharide (LPS) to induce inflammation. Bilobol appeared to exhibit antitumor effect via inhibiting the RhoA/ROCK signal transduction during the anti-inflammatory response [28].

Fucoidan, a sulfated polysaccharide isolated from brown algae, has been applied as an anti-cancer drug for hundreds of years in Chinese medicine. The results from Zhu et al. revealed that fucoidan had the capacity of antitumor partially through inhibiting the proliferation of HCC cells, although it is unable to repress the angiogenesis induced by HCC [29]. In another study, fucoidan displayed the antimetastatic efficacy on HCC cell lines via upregulating p42/44 MAPK-dependent NDRG-1/CAP43 pathway. Also, fucoidan was found to protect against bile acid-induced hepatocyte apoptosis. This ability suggested fucoidan presented a potent therapeutic agent for HCC treatment [30].

Telekin is a eudesmane-type sesquiterpene lactone extracted from the natural plant *Carpesium divaricatum*, which presents strong antiproliferative activity in cancer cells. Zheng et al. found that telekin promoted HCC cells apoptosis by activating the mitochondria-mediated apoptotic pathway [31].

Gigantol is a phenolic substance derived from the genus Dendrobium. Chen et al. investigated gigantol efficacy on liver cancer cells and the results suggested gigantol inhibited cells expansion and induced apoptosis in HepG2 cells through PI3K/Akt/NF-kappaB signal transduction [32].

The endoplasmic reticulum (ER) stress and unfolded protein response (UPR) play critical roles in the modulation of cell fate. The two factors even could become potent targets and provide support for the development of antineoplastic agents. Celastrol, one of the triterpene compounds derived from herbal medicine, exerts antitumor effects on various malignancies. Ren et al. demonstrated that for HCC cells, exposure to celastrol led to the sensitivity of the intrinsic apoptotic pathway, at least partly through ER stress and the UPR. Moreover, celastrol was found to repress H22 tumor growth in murine syngeneic model studies by inducing ER stress and apoptosis. These data suggested that targeting ER-stress/UPR was an efficient way for celastrol becoming a potent drug for HCC therapy [33]. Cytisine, a quinolizidine alkaloid, also a major bioactive constituent purified from the *Sophora alopecuroides* L. It was reported to exhibit inhibitory effects in treating liver cancer by inducing the ER stress-mediated apoptotic pathway through activating CHOP, JNK, and caspase-4 signaling pathways in liver cancer cells. This phenomenon suggested a novel target compound potentially to treat liver cancer [34].

RA-XII, a naturally occurring compound originated from Chinese herbal medicine Rubia yunnanensis, possesses activities of anti-inflammatory and antitumor. Song et al. revealed that RA-XII accelerated apoptosis and repressed protective autophagy via signaling pathway AMPK/mTOR/P70S6K in HepG2 cells, suggesting RA-XII, a cyclopeptide, provides the therapeutic support for potentially being an autophagy inhibitor drug in the therapy of hepatic tumor [35].

There are many bioactive compounds from Chinese medicine, which are also one part of daily diet. For example, *Bullacta exarata* is widely used as a part of normal diet in Asia, and also it is an agent with liver- and kidney-nourishing functions. One polysaccharide conjugate BEPS-IA was extracted from *B. exarata*. Liao et al. reported that BEPS-IA exerted a potent inhibition in HepG2 cells growth in a concentration-dependent manner via inducing apoptosis and blocking cell cycle. Furthermore, it was corroborated that this effect was involved in downregulation of Bcl-2, upregulation of p53, p21 and Bax, suggesting that BEPS-IA may be a new dietary drug for HCC obtained from herbals and shed light on getting a deeper understanding on the action mechanisms [36]. Diosgenin is a major bioactive component of Dioscoreaceae plants including yam, which is commonly prescribed in Chinese medicine, and a common vegetable all over the world. Diosgenin remarkably repressed the proliferation of several HCC cell lines in a dosage-dependent manner. Deeper investigation reported the apoptosis and cell cycle G2/M arrest were involved in the inactivation of Akt, activation of the caspase cascades, and upregulation of p21 and p27 expression. These results suggested that diosgenin may serve potentially as a novel antiliver cancer dietary supplement [37]. *Armillaria mellea* (*A. mellea*) is a honey mushroom, which is currently often consumed worldwide as a dietary supplement. Armillarikin was purified from *A. mellea*, which is an important component of Chinese medicine "Tianma." Chen et al. investigated the cytotoxicity of armillarikin against HCC cell lines such as Huh7, HA22T, and HepG2 cells. Armillarikin treatment induced apoptosis that was mediated by ROS and accompanied by the collapse of mitochondrial and activation of caspase-8 and -3 in cancer cells, suggesting the potential of armillarikin serving as an potent antihepatoma drug [38]. Corosolic acid analogue (CAA) is a triterpenoid saponin isolated from *Actinidia valvata* Dunn (Actinidiaceae), a kind of well-known fruit. The study investigated the antiproliferation and inducing apoptosis effects of CAA in three hepatoma cell lines. The data showed for the first time that CAA inhibited expansion of liver cancer cell lines and induced G1 phase arrest. Moreover, proapoptotic effect of CAA was mediated by the activation of TNF-α, caspases, and mitochondrial pathway [39].

1,6,7-trihydroxyxanthone (THA) is an active small molecule purified from *Goodyera oblongifolia.* The compound was discovered to strongly inhibit cancer cell proliferation and induced apoptosis in hepatoma carcinoma cells partially mediated by the repression of Bmi-1 and activation of miR-218 [40].

An active ingredient cordycepin was extracted from "Dong Chong Xia Cao." It has been implicated in regulating multiple physiological actions especially antitumor effects. Yao et al. revealed that cordycepin might contribute to tumor progression, EMT, migration, and invasion inhibition in HCC by suppression of signaling pathways E-cadherin and integrin/FAK. Hence, cordycepin is a supplementary candidate or therapeutic agent for preventing liver tumor expansion [41].

Norcantharidin (NCTD), a small-molecule antitumor drug originated from small animal *blister beetle*, has been currently applied as a potent antineoplastic agent for several kinds of cancers including HCC. The expression of FAM46C, which has been firstly reported as a tumor suppressor for multiple myeloma, was demonstrated to enhance with NCTD administration. FAM46C, a tumor inhibitor for HCC, was important for proapoptotic effects and antiproliferation of NCTD

[42]. Another study investigated the mechanism of NCTD-induced apoptosis in HepG2 cells, which indicated that NCTD could reverse the methylation state of RASSF1A gene and recover its expression, providing the theoretical information for further development in clinical application [43]. Also, Zhang et al. found in multiple HCC cell lines that NCTD could induce transcriptional repression of Mcl-1 and significantly enhance ABT-737-triggered cell viability inhibition and apoptosis [44].

Bufalin is the major bioactive constituent of the Chinese medicine Chansu, which is presently employed in clinical practice for cancer therapy. A number of groups have investigated the therapy efficacy of bufalin on hepatoma, either in vivo or in vitro, to explore the therapeutic potential of the drug. Qiu et al. reported that bufalin exhibited considerable antitumor activities in liver cancer cell lines HCCLM3 and HepG2 and the underlying mechanism might be related to the repression of signaling pathway AKT/GSK3β/β-catenin/E-cadherin [45]. Tsai et al. demonstrated that bufalin led to autophagic cell death and G2/M cell cycle phase arrest in SK-HEP-1 HCC cells via activating AKT/mTOR signal transduction pathway [46]. Another group reported that bufalin exerted remarkable antiproliferative activity and apoptosis induction in Huh-7 and HepG-2 cancer cells. Further study supported the prosurvival role of bufalin-induced autophagy when the autophagy pathway was retarded with specific chemical inhibitors, indicating a promising therapeutic approach for HCC therapy combining bufalin with a specific autophagy inhibitor [47].

In searching for active antihepatoma ingredients from *toad venom*, which is a frequent prescription applied in HCC treatment, Zhang et al. discovered that arenobufagin, a bufadienolide derived from toad venom, had prominent anticancer capacity against HepG2 cells and the corresponding multidrug-resistant cells, namely HepG2/ADM. They illuminated the molecular mechanisms of arenobufagin, which involved crosstalk between autophagy and apoptosis through PI3K/Akt/mTOR pathway suppression. Consequently, these findings contributed to the development of arenobufagin into a chemotherapeutic agent in liver cancer treatment [48]. Another compound, namely hellebrigenin, which was also isolated from *Venenum bufonis*, was found to significantly repress HepG2 cell viability and colony formation. Further exploration revealed the cytotoxicity of hellebrigenin in HepG2 cells and underscored the antihepatoma activity of hellebrigenin as an active component of *Venenum bufonis*. Hellebrigenin induced DNA damage, triggered cell cycle arrest, and subsequently initiated mitochondrial apoptosis. Moreover, Akt was found to take a role in cell cycle and apoptosis modulation induced by hellebrigenin. The findings showed the potential of hellebrigenin used as a chemotherapeutic drug for future HCC clinical application [49].

2.2. Functional roles of Chinese medicine extracts and fractions in liver cancer

Asparagus is not only consumed in daily diet but also employed as an agent in Chinese medicine for multiple types of malignancies therapy. An extract from asparagus, asparagus polysaccharide, has been confirmed to be the major bioactive constituent of asparagus in the respect of antitumor as well as immunity-enhancing activities. In clinical practice, it has been used in a number of malignancies treatment [50]. Weng et al. applied tumor-bearing rat model to systemically evaluate the toxicity and antitumor activity of asparagus polysaccharide and

asparagus gel-like material. The results showed a certain tumor inhibitory effect of them via promoting cell apoptosis and suppressing tumor angiogenesis when given as transarterial chemoembolization (TACE) therapy. Meanwhile, it exerted the antihepatoma activity with lower toxic effects as well as reduced kidney and liver functional damage, highlighting its chemotherapeutic potential in clinical application for future liver cancer TACE therapy [51].

Ganoderma lucidum polysaccharides (GLPS) have been exploited as folk Chinese medicine for their properties of immunomodulation and tumor prevention [52]. Li et al. measured the efficacy of GLPS on liver cancer cells in hepatoma-bearing mice model and effectively suppressed the tumor growth. The possible molecular mechanism may be related with an augment of the ratio of regulatory T cell (Treg) to effector T cell (Teff), which is caused by the augment of miR-125b, a predicative marker of poor prognosis and aggressiveness of liver cancer [53].

In China, *Trametes robiniophila Murr* (Huaier) has recently been used as Chinese medicine in China. It has a great clinical effect as adjuvant therapies in the treatment of HCC. Shan et al. investigated the functions of Huaier on HCC cells and confirmed that HCC growth could be restrained by Huaier through downregulation of yes-associated protein 1 (YAP1) [54].

Ampelopsis sinica root (ASR) is a well-known hepatoprotective Chinese medicine. Wang et al. explored whether ethyl acetate extract from ASRE had the antihepatoma activity both in vitro and in vivo. The findings showed that ASRE had prominent antihepatoma activity, which possibly involved the decreased regulation of inflammatory cytokines such as cyclooxygenase-2, 5-lipoxygenase and FLAP, augment of p53 protein expression and the ratio of bax/bcl-2, caspase-3 activation, as well as survivin repression. Moreover, ASR was found to be nontoxic on normal cells, suggesting that it may serve as a potential therapeutic agent for HCC treatment [55].

An extract of *Stellerachamaejasme* L. (ESC) had been confirmed as a potential antitumor extract of Chinese medicine. Liu et al. tested that the suppressive effects of ESC on propagation and epithelial mesenchymal transition (EMT) in liver cancer cells were associated with miR-107. The findings indicated ESC retarded HCC expansion and metastasis by regulating the expression of microRNAs and their according target genes [56].

Cnidium monnieri (L.) Cusson (CME) is a frequently used Chinese herbal medicine that treats gynecological diseases and carbuncles. A recent study showed the cell cycle alteration and apoptosis of HepG2 (wildtype p53) and Hep3B (p53null) by ethanol extract of CME, suggesting that CME induced G1 arrest and apoptosis via the Akt/GSK3β signaling pathway [57].

Astragalus membranaceus and Salvia miltiorrhiza are medical plants that have been applied for thousands of years in the treatment of liver diseases. According to previous researches, it has showed that these two herbs and their extracts own the ability to inhibit the development liver cancer. Rui et al. investigated that the compound astragalus and salvia miltiorrhiza extract (CASE) could repress diethylinitrosamine-induced hepatoma in rat model via the inhibition of fibrosis and PAI-1 mRNA transcription, indicating the possibility of being development as antihepatoma agents in preventing and treating human liver cancer [58].

Salvia chinensis Benth has been traditionally exploited for several centuries since old times to treat malignant diseases including HCC. In a study, total flavonoids isolated from *Salvia chinensis* Benth were shown to own the capability of inducing HCC cell apoptosis both in vitro and in vivo, which appeared to be implicated in the suppression of NF-κB activity [59]. *Coptidis rhizoma* has been used in clinical practice for tumor treatment in Chinese medicine, and recent experiments in our laboratory have supported its employment in tumor treatment. Zhu et al. examined the anticancer efficacy of *Coptidis rhizoma* aqueous extract (CRAE) on HCC cells and found the alterations of miR-21 and miR-23a after treatment with CRAE. The results suggested that CRAE targeted the miRNAs in hepatoma cells [60]. Wang et al. found that CRAE could remarkably downregulate Rho/ROCK signal transduction, then finally interfere MHCC97-L cell migration [61]. As we know, angiogenesis is an important factor, which is beneficial for tumor expansion. Tan et al. confirmed that antiangiogenic effect of CRAE on HCC was partially dependent to an eEF2-driven pathway [62]. All these findings supported the potential application of CRAE in HCC therapy.

Prunella vulgaris (PV) is a small tree that has been employed clinically for thousands of years in Asia to treat herpetic keratitis. According to previous researches, it has showed PV could repress TPA-induced activation of MMP-9 and suppress hepatoma cells migration and invasion. Data suggested that by modulating multiple signaling pathways, PV modified the metastatic microenvironment of HCC. PV thus may provide useful information for systemic therapies of HCC [63].

Ethyl acetate extract (EAE) of *Euphorbia helioscopia* L. played a critical role in repressing tumor cell proliferation, apoptosis, invasion, and metastasis in vitro. Meanwhile, Cheng et al. found that change of expression of cyclin D1, Bcl-2, Bax, MMP-9 by EAE may be associated with inhibition of tumor growth, induction apoptosis, and suppression of tumor metastasis and invasion in HCC xenografts [64].

Some Chinese medicine scholars have indicated that endogenous wind-evil acted as a critical role in tumor metastasis. On the basis of this, the agent of dispelling wind-evil could serve as a suppressor for cancer metastasis and poor prognosis. Yan et al. observed that scorpion-medicated serum could restrain proliferation, induce apoptosis, as well as inhibit the capacity of migration and invasion in vitro. Further experiments in HCC tumor-bearing metastasis mice models showed that water decoction of scorpion blocked tumor growth and metastasis. More importantly, these results suggested that scorpion, as an important wind calming drug, could inhibit the metastasis and invasion of liver cancer cells especially through epithelial-mesenchymal transition (EMT) reversal, thereby providing a possible potential approach to preventing HCC metastasis [65].

Actinidia chinensis Planch root extract (acRoots) has been shown to inhibit cell proliferation in numerous cancer cells. Hou et al. used acRoots to treat HCC cells and observed the distinct effects of acRoots on cell proliferation, cell cycle arrest, and apoptosis. Furthermore, the mechanism underlying these activities was attributed to LAMB3-mediated proliferation suppression and S-phase cell cycle arrest in HepG2 cells [66]. He et al. studied the mechanism in

the extent of metabolic alterations. The data showed that acRoots could remarkably inhibit cholesterol metabolism through a PCSK9-mediated signaling pathway, which in turn limited the nutrients production that was essential for the proliferation of cancer cells [67].

Ethanol extract of root of *Prunus persica*, which is an important ingredient in Chinese medicine prescription, exhibited antitumor effect in liver cancer. Scholars recently reported that *Prunus persica* could repress cell growth in a time and dose-dependent fashion, causing sustained M/G2 phase arrest as well as notably suppressing the migration of HepG2 cells and the expression of extracellular matrix metalloproteases, MMP3 and MMP9 [68].

Realgar (As_4S_4), one of the most useful mineral drugs in Chinese medicine, has been employed in clinical therapy as a potential agent for cancer therapy. However, due to its low solubility and subsequent poor bioavailability, it is difficult to achieve the effective blood medicine dose unless with high dosage of realgar and long period of treatment. A recent study explored realgar transforming solution (RTS) and found the strong antihepatoma activity of RTS via inducing ROS [69].

2.3. The role of Chinese medicine composite formulae in regressing liver cancer

Huang-lian-jie-du-tang (HLJDT) is oriental medicinal formulation known to possess anti-inflammatory activity. The prescription has been well documented for thousands of years and used for liver protection in Asian community [70]. Recent researches have postulated HLJDT as a regimen for cancer treatment, particularly hepatoma. Hsu et al. found that HLJDT might have an effect on human liver cancer cell lines, Hep G2 and PLC/PRF/5. The results showed that HLJDT significantly triggered cell cycle arrest and contributed to the mitochondrial apoptotic pathway by reducing the level and activity of NF-κB, which suggested that HLJDT might be a promising chemotherapeutic agent without causing cytotoxicity to normal cellular environment [71]. Wang et al. examined the suppressive efficacy of HLJDT on the liver cancer expansion and found that involvement of eEF2 inhibition might be the key mechanism mediating the inhibitory effect of the formula [72].

Yiguanjian (YGJ), a classic liver-YIN tonifying herbal formula, was established by ancient Chinese medicine practitioner Wei Zhixian in the Qing Dynasty (AD 1722–1772). Researchers optimized the prescription of YGJ on the basis of modern principles in clinical practice of Chinese medicine and then evaluated the antitumor activity of modified YGJ (MYGJ) on Bel-7402 human liver cancer cells. These data showed that MYGJ could interfere proliferation suspension and induce anoikis in cancer cells. The mechanisms underlying the actions of MYGJ might involve in inhibiting the phosphorylation and expression of p38 MAPK, and subsequent regulating intrinsic and extrinsic pathways of apoptosis [73].

Pien Tze Huang (PZH) is an extensively employed prescription in the treatment of multiple malignancies and has possible therapeutic effects in clinical therapy for HCC. Qi et al. aimed to elucidate the efficacy of PZH on the proliferation and apoptosis of liver cancer cell lines and demonstrated PZH could effectively inhibit cancer cell proliferation and induce apoptosis in Bel-7402 HCC cells by upregulating miR-16, which has been verified as tumor suppressor, suggesting a novel potential therapeutic for HCC patients [74].

Sini-San (SNS) has been employed for the treatment of various types of liver disease. This formulation comprises four prescriptions of Chinese herbal medicine and was first described in "Shanghan Lun (Treatise on Cold Damage Disorders or the Treatise on Cold Injury)," established by one of the most famous ancient Chinese physicians, Zhang Zhongjing (150–219 AD). SNS has shown significant inhibition on tumor growth in HepG2 xenograft model. Lin et al. elucidated the molecular mechanism by which SNS exerted an antimigratory and anti-invasive effect on HBx-activated liver cancer cells. These results showed that SNS suppressed invasiveness and metastasis in HCC cells via multiple signal transduction pathways including downregulating PI3K/Akt, decreasing MAPK and IκB signaling, inhibiting NF-κB and AP-1 activity, and reducing MMP-9 expression. Thus, SNS might be helpful to interfere the invasion and metastasis of HCC [75].

Songyou Yin (SYY), a composite formula, showed efficacy to repress tumor proliferation, metastasis, and recurrence. An interesting study explored that SYY combining with moderate swimming has potent effect on retraining tumor growth and metastasis mainly via enhancing immune function [76].

Niu-Huang-Shen (NHS) has been accepted and used in China for a long time with its various effects such as antipyretic, anti-inflammatory, and vasodilatation effects. It was showed that NHS inhibited cell cycle arrest, induced cell apoptosis, and then repressed cell proliferation and invasion, probably through the significant suppression of Yes-associated protein (YAP) expression. NHS may have the therapeutic potential for treating HCC more effectively [77].

Shuihonghuazi formula (SHHZF) has been employed for early stage of liver cancer in clinical therapy for a long time; a study was designed to investigate potent effects of SHHZF on hepatoma and its metabolomic profiles. The results elucidated that SHHZF exerted inhibitory effects against liver cancer by adjusting the activities of PE N-methyl transferase, lysophospholipase D, methylenetetrahydrofolate reductase, and lysophospholipase [78].

3. Chinese medicine as a complementary treatment of liver cancer

Chinese medicine is appreciated for its 5000-year-old history and still holds a prominent position in primary health care in China. Chinese medicine could complement Western medicine by using modern techniques; thus, increasing interests in Chinese medicine has been observed over the Western world. In Chinese medicine, a wide range of ingredients have been proven to achieve various effects in cancer therapy, including alleviating the toxicity to human body, retraining tumor metastasis and recurrence, enhancing chemo- or radio-therapeutic effects, and subsequent improving the general status of patients and extending their survival time.

Long-term food restriction and diarrhea may be an adverse factor for liver cancer. Jian-pi-jie-du decoction (JPJD) could improve the quality of life of hepatoma subjects, in particular, the symptoms of diarrhea and decreased food intake. A research indicated JPJD could improve the condition of tumor-bearing rats, which were pretreated with diarrhea and food restriction by increasing ABCC2 expressional level and downregulating the OATP1B2 in liver normal tissues while downregulating ABCC2 as well as upregulating OATP1B2 in cancer tissues [79].

In terms of radioprotective and radiosensitizing functions of Chinese medicine, a series of concerning studies have been conducted. Numerous Chinese medicine agents have been confirmed to strengthen the therapeutic gain of radiotherapy by the way of serving as radioprotectors for healthy cells or as radiosensitizers for cancer cells [80, 81]. Botanical agents are comprised of multiple phytochemical compounds that may work synergistically or even individually, not only exhibiting favorable therapeutic effects, but also with safety profiles and lower toxicity [82].

Ganoderma lucidum polysaccharide (GLP) is well known for its various pharmacologic properties including antitumor effects [52]. A study recently demonstrated that GLP treatment may augment growth inhibition and apoptotic death of HepG2 cells, which induced by radiation, and revealed the regulatory role of Akt signaling pathway for GLP-mediated radiosensitivity in HCC cells exposed to radiation [83].

Kou et al. investigated the radiosensitizing effects of ultrafiltration extract of Radix Angelicae Sinensis-Radix Hedysari (RAS-RH) in human hepatoma cells. The results reported that the RAS-RH significantly enhanced the radiosensitivity of H22 cells of 12C6+ heavy ion radiation. Further study explored the underlying mechanism of radiosensitization, which is to increase caspase-dependent apoptosis via reducing surviving expressional level, suggesting a promising potent radiosensitizer [84].

Zhang et al. demonstrated that a flavonoid dihydromyricetin (DHM) exerted anticancer activity against hepatoma cells as well as xenotransplanted tumors in nude mice by activating the p53-dependent apoptosis pathway. And best of all, DHM was indicated to play a prominent role when administered in combination with cisplatin [85]. In this case, DHM could be an ideal anticancer drug with minimal side effects because it can alleviate cytotoxicity caused by cisplatin in normal liver cells.

Some studies investigated the adjunctive role of bufalin in reversal chemoresistance in the treatment of liver cancer. The Akt activation triggered by sorafenib is regarded to be responsible for this resistant phenomenon. Zhai et al. investigated that bufalin had the ability of reversing both inherent and acquired resistance to sorafenib via the IRE1 pathway in an ER-stress-dependent manner. These data warranted further studies to examine the utility of bufalin in combination with sorafenib as a first- or second-line treatment after sorafenib alone gains failure in advanced liver cancer [86]. Fluorouracil (5-FU) is a type of anticancer chemotherapeutics, which has been used for 40 years in clinical practice. A research confirmed the reversal effect of bufalin on drug resistance in a moderate multidrug resistance cell line Bel-7402/5-FU. They found Bufalin could block the cell cycle at G_0/G_1 phase, induce apoptosis through an increase of Bax/Bcl-xL ratio, inhibit the drug efflux pump activity via downregulation of MRP1, and reduce the expression of thymidylate synthase in vitro. All these data revealed that in Bel-7402/5-FU cells, the combination of bufalin with cytotoxic drugs could considerably reverse the MDR through multiple pathways including cell cycle arrest, apoptosis induction, etc., indicating an effective strategy for the chemotherapy of HCC [87].

Xu et al. investigated the efficacy of drug combination of luteolin and 5-FU on the proliferation of HepG2 and Bel-7402 cells. The data showed that luteolin synergized 5-FU at different dose ratios and then exerted the antitumor effects against HCC cells. Potential mechanism for

synergistic effects may be associated with apoptosis and 5-FU metabolism, as evidenced by the increased bax/bcl-2 ratios, upregulated p53 expressions, and induced PARP cleavage [88].

ADCX, a natural cycloartane triterpenoid isolated from *Cimicifugae* rhizome, impaired autophagic degradation by inhibiting lysosomal cathepsin B expression in multidrug resistant cell line, namely HepG2/ADM, which consequently lead to apoptosis, suggesting that an active constituent from Cimicifugae rhizome could overcome multidrug resistance in hepatoma cells by the role of persistent Akt activation in inhibition of autophagic degradation [89].

Arsenic trioxide (As_2O_3) with high doses is employed to treat solid tumors and acute promyelocytic leukemia, which mostly induce toxic side effects to healthy cells. Andrographolide is a kind of Chinese medicine that exhibits various effects against diseases such as anti-inflammatory, antivirus, antitumor, and so on. Duan et al. demonstrated that andrographolide enhanced As_2O_3-induced apoptosis in a caspase-3-dependent manner via downregulation of EphB4 in HCC cells. These findings suggested that lower concentrations of As_2O_3 in combination with andrographolide could be used as chemotherapy for HCC with the potential to minimize the adverse events from As_2O_3 treatment alone [90].

The aqueous extract of *Solanum nigrum* (AE-SN) is an important constituent in some Chinese medicine formulae used in the treatment of cancer. Wang et al. explored the antitumor effect of AE-SN in combination with a normal chemotherapeutic drug, namely doxorubicin or cisplatin, in HCC cell lines Hep3B and HepJ5. The results indicated the integrated treatment with AE-SN-potentiated doxorubicin and cisplatin-induced cytotoxicity through the cleavage of caspase-7 and accumulation of microtubule-associated protein-1 light chain-3 A/1B II (LC-3 A/B II), which were involved in autophagic and apoptotic cell death, respectively. Thereby, this combinatorial strategy of AE-SN and cisplatin or doxorubicin may be exploited to be a candidate regimen to treat HCC patients [91].

A recent research was performed to explore the combination effect of Huaier aqueous extract and chemotherapeutic agent cisplatin or rapamycin. The findings showed that Huaier had the capacity of activating mTOR signaling, which contributed to the enhanced cancer cells sensitivity to chemotherapeutics in response to Huaier administration. Huaier, thus, can potentially be used in integrated chemotherapy with rapamycin or cisplatin for liver cancer therapy [92].

Cinobufacini, a mixture of a number of components in Chinese medicine, has been used extensively for HCC therapy with strong apoptosis-inducing activity. Xia et al. used a combination of doxorubicin with cinobufacini to achieve tumor-suppression efficiency and found the combination group had a more considerable apoptotic effect by affecting proteins and RNA of apoptosis-related elements, such as Bcl-2, Bax, Bid, and cytochrome C. Consequently, cinobufacini in combination with chemotherapeutic agents might be a new strategy to improve the treatment effect for HCC patients [93].

Shufeng Jiedu Capsule (SFJDC) has been widely used due to its various pharmacological actions such as anti-inflammation, antibacterial, antiviral, and antitumor. Recently, scholars used combination of SFJDC with doxorubicin to treat liver cancer cells and further explored the underlying mechanisms of SFJDC as well as its constituents in vitro. The data showed that the combination group induced more considerable apoptosis and invasion and migration suppression than control group by targeting NF-κB, Akt/mTOR, and mitochondrial signaling pathways [94].

Dahuang zhechong pill (DHZCP) is one of the most famous prescriptions from an ancient Chinese medical classic "Jin Kui Yao Lue (Essential Prescriptions from the Golden Cabinet)." DHZCP is officially recorded in the Chinese Pharmacopeia and is commonly used for clinical practice of hepatoma. Wu et al. found that inhibitory growth of doxorubicin-resistant HCC subcutaneous xenografts in nude mice was achieved by DHZCP, and apoptosis promotion was accelerated by doxorubicin. The reversal of doxorubicin resistance by DHZCP was related with energy metabolism decline and regulation of proapoptotic proteins expression [95].

4. Discussion

Accumulating researches have demonstrated that Chinese medicine is a promising substitute for therapy of liver cancer. Furthermore, increasing scholars starts to pay attention to clinical studies of Chinese medicine. For example, gambogic acid (GA), a naturally occurring compound from ancient China, has been demonstrated efficient antineoplastic activity in a number of malignancies. More importantly, it has entered phase II clinical trials. A team ?found GA might lead to oxidative stress and subsequently induce apoptosis in hepatoma cells through interacting with TrxR1. Thus, targeting TrxR1 by GA disclosed a previously unrecognized mechanism underlying the biological action of GA and provides useful information for further development of GA as a potential agent for cancer therapy [96]. On the other hand, the theory of "Jianpi Huayu Therapy" (JPHY) was rooted from "Jin Kui Yao Lue." According to the selection criteria, Zhong et al. recruited a total of 120 patients in a randomized trial, aiming to compare the curative outcome and safety profile of surgery in combination with "Jianpi Huayu Therapy" HCC treatment to surgery alone. The patients in treatment group received the basic prescription based on JPHY. The results showed that hepatectomy combined with JPHY was more effective with reducing postoperative metastasis and recurrence and prolonged overall survival of HCC patients [97]. JQ1, one of the bromodomain and extra-terminal domain (BET) inhibitors, has been emerged as a novel agent candidate for cancer treatment in clinical research. Nevertheless, a number of solid cancers are resistant to BET inhibitors. The results from a group showed that oridonin synergistically increased JQ1 capacity of inhibiting HCC cell survival, and considerably enhanced JQ1-caused apoptosis in HCC cells and in HCC cancer stem-like cells. Furthermore, they demonstrated that oridonin distinctly augmented the sensitivity of JQ1 via downregulation of the level of multiple antiapoptotic proteins, including Bcl-2, Mcl-1, and x-linked inhibitor of apoptosis, suggesting that the combination treatment of JQ1 and oridonin could be further pursued for clinical application and it was expected to provide a rational for HCC tumor prevention [98].

Collectively, the aforementioned findings showed the potential efficacy of Chinese medicine on numerous types of cancer, either alone or in combination with conventional treatment of method such as surgery, chemotherapy, or radiation. In particular, as stated above, when integrated with chemotherapy or radiotherapy, Chinese medicine may serve as complementary drugs strongly enhancing the positive effects or reducing the negative events induced by radiochemotherapy. However, in comparison with a great deal of laboratory researches, clinical trials still remain poor, which limits the wide application of Chinese medicine throughout the world.

5. Conclusion

Chinese medicine is increasingly emerging as a novel curative choice for liver cancer. This retrospective review systemically introduced and evaluated the functional roles of Chinese medicine in treating liver cancer. Chinese medicine has potentially exerted efficient anticancer properties. For example, liver cancer progression can be repressed by active constituents derived from Chinese medicine through multiple pathways. The specific network with regard to the potential therapeutic targets for liver cancer treatment was constructed (**Figure 1**). The detailed relationships between biological factors and refined extracts could be directly visualized in **Figure 1**. Moreover, composite formulae as promising curative are increasingly indispensable in current clinical practice. As summarized in **Table 1**, formulae potentially employed in practice were studied in laboratory and the regulatory mechanisms for the treatment of liver cancer have been showed clearly. Also, Chinese medicine may serve as adjuvant agents in surgery as well as in combination with conventional radio- and chemotherapy, to decrease the adverse events or enhance the treatment outcome. Taken all together, Chinese medicine possesses the potential in liver cancer treatment, and rational application in clinical therapy needs to be warranted in the future.

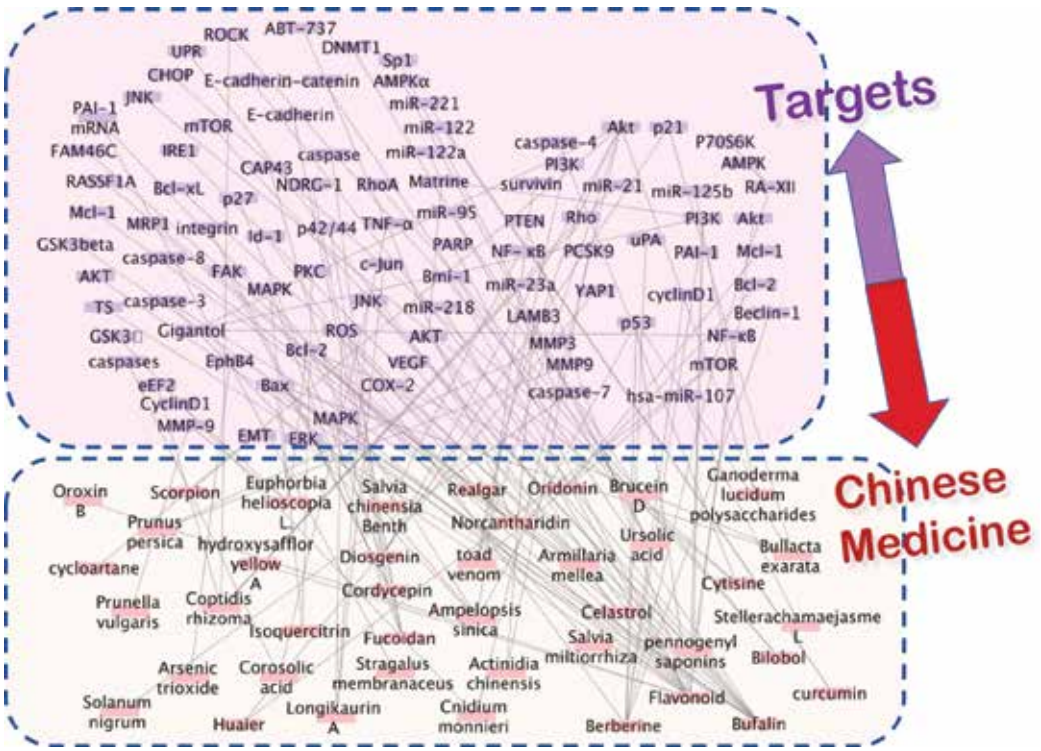

Figure 1. Target identification of Chinese medicine-derived compounds and extracts for liver cancer. Literature mining in PubMed with "Chinese Medicine" integrated with "liver cancer" was performed. All filtered data during the last 5 years were imported into a professional software Cytoscape for the establishment of the analysis of network pharmacology. The top five influential molecules including Akt, Bax, Bcl-2, mTOR, and PI3K could be figured out.

Name	Functions	Ref.
Huang-lian-jie-du-tang	Cell cycle arrest, induce mitochondrial apoptotic pathway, inhibit HCC cell proliferation, suppress growth and angiogenesis in xenografted murine model	[71, 72]
Yiguanjian	Interfere proliferation suspension and induce anoikis in cancer cells	[73]
Pien Tze Huang	Inhibit cancer cell proliferation and induce apoptosis	[74]
Sini-San	Suppressed invasiveness and metastasis in HCC cells	[75]
Songyou Yin	Repress tumor proliferation, metastasis, and recurrence	[76]
Niu-Huang-Shen	Cell cycle arrest, induce cell apoptosis, and cell invasion	[77]
Shuihonghuazi formula	Increase the uptake and utilization of linoleic acid and oleic acid, increase arachidonic acid-like substance content, and enhance organism immunity of liver cancer rats	[78]
Jian-pi-jie-du decoction	Improve the condition of tumor-bearing rats with the symptoms of diarrhea and decreased food intake	[79]
Cinobufacini	Combination of doxorubicin with cinobufacini to achieve a more considerable apoptotic effect	[93]
Shufeng Jiedu Capsule	Combination of SFJDC with doxorubicin induced more considerable apoptosis and invasion and migration suppression	[94]
Dahuang zhechong pill	Inhibit growth of doxorubicin-resistant HCC subcutaneous xenografts in nude mice and accelerate apoptosis promotion integration with doxorubicin	[95]

A comprehensive screening among literature searched with "Chinese Medicine" combined with "liver cancer" was performed. Potential composite formulae for therapeutic of liver cancer were screened out and corresponding possible action mechanisms were summarized.

Table 1. Summary on Chinese medicine composite formulae potentially used for liver cancer treatment.

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Section 5

Biology and Immunology of Liver Cancer

Chapter 8

Interaction of Mitochondrial and Epigenetic Regulation in Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is a pathology preceded mainly by cirrhosis of diverse etiology and is associated with uncontrolled dedifferentiation and cell proliferation processes. Many cellular functions are dependent on mitochondrial function, among which we can mention the enzymatic activity of PARP-1 and sirtuin 1, epigenetic regulation of gene expression, apoptosis, and so on. Mitochondrial dysfunction is related to liver diseases including cirrhosis and HCC; the energetic demand is not properly supplied and mitochondrial morphologic changes have been observed, resulting in an altered metabolism. There is a strong relationship between epigenetics and mitochondrion since the first one is dependent on the correct function of the last one. There is an interest to improve or to maintain mitochondrial integrity in order to prevent or reverse HCC; such is the case of IFC-305 that has a beneficial effect on mitochondrial function in a sequential model of cirrhosis-HCC. In this model, IFC-305 downregulates the expression of PCNA, thymidylate synthase, HGF and its receptor c-Met and upregulates the cell cycle inhibitor p27, thereby decreasing cell proliferation. Both effects, improvement of mitochondria function and reduction of tumor proliferation, suggest its use as HCC chemoprevention or as an adjuvant in chemotherapy.

Keywords: hepatocellular carcinoma, cell cycle, cell proliferation, mitochondria, epigenetics, IFC-305

1. Introduction

Hepatocellular carcinoma (HCC) represents 80% of the primary liver cancer and, in minor proportion, bile duct cancer and angiosarcoma of the blood vessels in the liver, but all of them have a poor prognosis. HCC is a major cause of cancer-related deaths globally. The incidence of HCC is increasing and has been rising in the last few decades [1]. The HCC is a complex pathology associated in 80–90% with chronic liver diseases like cirrhosis of diverse etiologies. Cirrhosis is a chronic degenerative disease of the hepatic parenchyma characterized by an inflammation process that leads to liver fibrogenesis. This process induces the loss of liver architecture and a diminution of functional parenchyma, which over time changes the environment of the cells resulting in chromosomal instability. The cause of cirrhosis transformation into HCC is not well known, but chromosomal instability could be an important factor for HCC generation in cirrhotic patients. The main problem of this pathology is the lack of early detection, recurrence of tumors following resection [2], and there are no effective therapies. To understand this complex pathology, it is convenient to have some knowledge of the structure and functions of the liver. Therapeutic options for HCC are very limited, and the incidence is very similar to the death rate per year. Only in the early stage of the disease, there are some approved therapies such as tumor ablation, surgical resection, and liver transplantation, but in advanced stages, when most patients are diagnosed, these treatments are not recommended. There is an average of 5-year survival below 20% with these therapies [3]. In intermediate and advanced stage-HCC, the approved options are transcatheter arterial chemoembolization (TACE) and the multi-kinase inhibitor, sorafenib. TACE therapy could extend survival to 2 years [3]. Sorafenib extends survival of patients with advanced stage disease for only 3 months, and this medication causes considerable adverse effects and offers no symptom palliation [4]. There are other several clinical trial efforts focused on therapies involving multiple signaling pathways, most commonly related to tyrosine-kinase growth factor receptors, but they have inferior survival benefits and several adverse effects. Immunotherapy has demonstrated some efficacy, but, in general, molecular characterization to find effective treatments of HCC is needed.

The liver is the largest internal and heterogeneous organ in the body constituted by different kinds of cells like hepatocytes, endothelial cells, cells of the bile duct, Kupffer cells, hepatic stellate cells (HSC), oval cells and pit cells [5]. The liver is an organ highly irrigated by the portal venous system and blood is distributed by the hepatic sinusoids and the hepatic artery [6]. About 80% of the liver cells are hepatocytes, and are epithelial cells that form cords with high metabolic activity and contain a complete set of organelles: mitochondria, peroxisomes, lysosomes, Golgi complex and a well-organized cytoskeleton [7]. The space between cords of hepatocytes and the endothelium is called the space of Disse. Endothelial cells constitute the wall of the hepatic sinusoids and are separated from the parenchymal cells by the space of Disse. They possess pores or fenestrae that permit the exchange of fluids [8]. These cells show endocytic activity and secrete several mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon, and nitric oxide as paracrine modulators. Kupffer cells are the fixed macrophages of the liver that can migrate along sinusoids. Their main function is an immunomodulatory one [9]. Pit cells are intrahepatic leucocytes with natural killer cell activity [10] and exert a cytotoxic activity toward tumor and virus-infected cells [11]. HSC, also known as lipocytes, fat storing cells, perisinusoidal cells, and vitamin A storing cells, are quiescent in normal conditions.

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When they are activated, they play an essential role in the synthesis and degradation of the extracellular matrix (ECM) proteins and fibrogenic cytokines, like hepatocyte growth factor (HGF), insulin growth factor (IGR), transforming growth factor-β (TGF-β), and, consequently, induce cirrhosis. Biliary epithelial cells participate in the formation of bile; they are transported to the bile ducts or Canals of Hering. These cells have the potential to become oval cells [7]. The cell-free hepatic tissue represents 20% of the liver volume and constitutes the ECM located in the Disse space. The ECM contains structural proteins like collagen of different types, glycoproteins, fibronectin, tenascin, laminin, entactin, and perlecan. Their function is to maintain the hepatic architecture and the organization of the entire organ. Hepatocytes contribute with 80–90% of the synthesis of liver collagen, which is degraded by metalloproteinases (MMPs) [12]. The liver has multiple functions needed for its own metabolism and for other organs; it participates intensely in the intermediary metabolism that occurs mainly in hepatocytes and is connected with the nutrients of the diet, reaching from the portal circulation, that is, in carbohydrates, proteins, and lipid metabolism. The liver also generates purines and pyrimidines for its own use and their distribution to other tissues in the form of adenosine, inosine, and hypoxanthine [13]. It also synthetizes and secretes plasma proteins and participates in the biotransformation of endogenous and exogenous compounds.

Previously, we have demonstrated that adenosine is a metabolic modulator of glucose and lipids in the liver and adipose tissue [14]. This molecule also modulates in vivo the energy charge in the liver [15]. The nucleoside adenosine is a substance with multiphysiological effects in different tissues, the central nervous system, and cardiovascular system; it is responsible for the modulation of the immune response and acts as metabolic regulator. Its action could be autocrine, paracrine, and endocrine; its metabolism is very active with a high turnover and a very short half-live. Adenosine presents circadian variations in the rat, which correlated with energetic homeostasis of the cell, modulation of membrane structure and function, cell proliferation, and genetic expression by regulating physiological methylation [16]. Exogenous adenosine administration to normal rats showed some pharmacological effects, like increased ATP levels simultaneous to a decrease in ADP and AMP, resulting in an increase of the energy charge of the liver [14]. Also, in the liver of fasted rats, adenosine induces an enhancement of glycogen synthesis [16] and an inhibition of fatty acid oxidation by inhibiting the extramitochondrial acyl CoA synthase and decreasing the plasma ketone bodies [17] These findings allowed us to demonstrate in vivo the Atkinson hypothesis of metabolism regulation by energy charge [18].

The redox state of the cell in different compartments, calculated by the NAD^+/NADH (NAD^+ and NADH nicotinamide adenine dinucleotide, oxidized and reduced) system, has been shown to be a key point in the control of metabolism [19]. Adenosine administration induces mitochondrial oxidation and promotes the oxidized state in the cytosol and mitochondria in the presence of fatty acid oxidation inhibition, which is induced by the nucleoside. It has been reported that adenosine modulates vasodilatation and vasoconstriction in the hepatic vessels controlling blood flow from the hepatic artery [20]. All these results observed in normal animals led us to test the effects of the nucleoside in several models of acute hepatotoxicity: one induced with ethanol [21], the second with cycloheximide, and the third with carbon tetrachloride (CCl_4). Although the toxic mechanism of each one is different, they yielded a similar response generating a fatty liver that was prevented by adenosine [21–23]. In this way, the nucleoside, through different mechanisms, protects the liver against acute toxicity.

Continuous acute hepatotoxicity results in chronic liver injury with subsequent cirrhosis, with accumulation of ECM proteins, mainly collagen type I [24], accompanied by a deficient degradation of deposited collagen [25]. These conditions will induce a change in liver architecture with loss of its function. This is a complex process, for which no effective treatment has been developed yet. To study the effects of adenosine in this process, a model of cirrhosis induced in rats with CCl_4 was developed, in which two conditions were tested: prevention during cirrhosis development and reversion once it is already established [26, 27]. The simultaneous administration of adenosine partially blocked the stimulated collagen synthesis induced by the hepatotoxin, maintained high levels of hepatic collagenase activity, resulting in 50% diminution of fibrosis [26]. The effect of the nucleoside was clearly observed also in the reversion model; it was tested in well-established cirrhosis after 10 weeks of CCl_4 administration. Five weeks after suspension of the toxin, animals were treated with saline or adenosine, the saline group increased the cirrhotic characteristics but the group of animals treated with the nucleoside revealed blocked fibrogenesis, increased collagen degradation and normalized collagen types ratio, promoted hepatocyte proliferation, accelerated normalization of liver function, and decreased oxidative stress. These results suggest adenosine as a potential therapeutic agent in the treatment of chronic hepatic disease.

The transfer of an interesting research finding to a clinical setting is complicated, but in collaboration with Dr. Francisco Hernández Luis from the National Autonomous University of Mexico's School of Chemistry, we prepared several adenosine derivatives that were tested in the CCl_4 induced cirrhosis. The aspartate of adenosine, named IFC-305, showed interesting results [28]; beneficial effects in structure and functional recovery were obtained with a fourfold lower dose of this adenosine derivative because it has a longer half-life. The hepatoprotective mechanism of IFC-305 on fibrogenesis was investigated by means of DNA microarrays analysis [29], showing that the expression of 413 differential genes deregulated in cirrhosis tended to be normalized by IFC-305 treatment. Fibrogenic genes, such as TGF-β, collagen type I, fibronectin I, increased their expression in cirrhotic groups, and IFC-305 diminished their expression supporting the antifibrogenic action of the compound. These results highly suggest a diminution of chromosomal instability. With the increased understanding in chromatin organization of the eukaryote genome at genetic and epigenetic levels and remembering the previously commented role of adenosine on physiological methylations, a possible epigenetic mechanism of the IFC-305 could participate in the obtained results. Global changes in DNA methylation, 5-hydroxymethylation and histone H4 acetylation were decreased in cirrhosis and after the IFC-305 treatment the normal values were recuperated. In contrast, the promoter of *Col1a1* gene is hypomethylated in cirrhosis but gains DNA methylation upon treatment with IFC-305, correlating with a decrease of Col1a1 transcript and protein level, showing that the treatment restores globally and specifically epigenetic modifications [30]. The microarray analysis also showed modification of immunity genes which where explored in the CCl_4 model; it was found that the IFC-305 compound reduced inflammatory cytokines and increased the anti-inflammatory ones like IL-10, supporting the modulation of the macrophage phenotypes M1 and M2 [31].

2. Hepatocytes proliferation in cirrhosis and cancer, modulation by IFC-305

The liver is an organ with regenerative capacity. Partial hepatectomy or diverse stimuli promote proliferation of parenchymal and non-parenchymal cells in order to recover the liver

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mass and architecture. This process is regulated by cell cycle proteins, cytokines, growth factors, and matrix remodeling [32].

In acute liver injury, there is a classic wound healing process in which inflammation triggers scar formation that is subsequently resolved to enable regeneration of the damaged hepatic parenchyma. However, when there is a chronic liver injury, the normal regenerative process is impaired, and instead a net deposition of fibrillar collagen is predominant [33].

Cirrhosis is characterized by a decrease in hepatocyte proliferation, in part, because liver cells have a limited regenerative capacity restricted by telomere length. After several rounds of replication, telomeres reach a critically short length that induces cell cycle arrest, senescence, and apoptosis of hepatocytes. Telomere shortening also activates DNA repair pathways leading to chromosomal fusions and instability [34]. During cirrhosis-activated HSC, inflammatory cells secrete proliferative and angiogenic cytokines that contribute to a proliferative condition milieu, including: HGF, vascular endothelial growth factor (VEGF), and IL-6 [33]. This proliferative milieu could stimulate the proliferation of altered hepatocytes carrying mutations of cell cycle checkpoint genes or could select genetically altered clones, promoting HCC [34].

Among the principal cell cycle checkpoints that are generally altered in HCC are the tumor suppressor p53 and Rb proteins. p53 is implicated in cell cycle control, DNA repair, apoptosis, and regulates different metabolic pathways [35, 36]. p53 is frequently mutated in HCC (28–50%) and core proteins from hepatitis B and C viruses can repress p53 activity [36]. The pRB protein is implicated in the progression from G1 into S phase. The Rb pathway is disrupted in more than 80% of human HCC [34]. Gankyrin binds Mdm2 promoting proteasomal degradation of p53 and pRb. Both gankyrin and Mdm2 proteins are frequently overexpressed in human HCC [34, 35]. p53 is also implicated in the stimulation of ATP production by oxidative phosphorylation (OXPHOS). p53 also decreases glycolysis and cellular reactive oxygen species (ROS) production by inducing a protein called TP53-induced glycolysis and apoptosis regulator (TIGAR). TIGAR blocks glycolysis by degrading fructose-2,6-bisphosphate. This inhibition redirects glucose-6-phosphate into the pentose phosphate pathway, which increases NADPH production increasing the antioxidant defenses. The inactivation of p53 should decrease OXPHOS and increase glycolysis and ROS production in cancer cells [37].

It has been demonstrated that IFC-305 is able to stimulate hepatocytes proliferation in CCl_4-induced cirrhotic liver through the upregulation of proliferating cell nuclear antigen (PCNA), HGF, and p53, with an increase in energy and preservation of mitochondrial function [38].

On the other hand, in a sequential model of cirrhosis-HCC induced by diethylnirosamine (DEN), IFC-305 caused a tumor reduction, and this protective effect was associated with decreased cell proliferation in the HCC stage. This effect was associated with a decreased expression of PCNA, thymidylate synthase, HGF and its receptor c-Met, and the induction of the cell cycle inhibitor p27. IFC-305 also induced a diminution of gankyrin expression contributing to restoring p53 protein expression to control levels [39].

How could the same compound IFC-305 have opposing effects on proliferation in normal versus transformed hepatocytes? These could be mediated partly by a differential expression of the HGF-c-Met pathway driven by IFC-305 treatment, and the dual role of HGF/c-Met in cirrhosis and liver tumorigenesis. HGF expression is restricted to cells of mesenchymal origin, whereas the receptor c-Met is expressed in epithelial and endothelial cells. HGF is implicated

in cell proliferation, survival, morphogenesis, cell motility, and metastasis. This pathway plays a critical role in tissue protection and regeneration. It has been used as a therapeutic agent in fibrosis of different organs. The protective actions of HGF are associated with promotion of cell proliferation, migration, and morphogenesis that would help tissues reorganization [40]. Its protective role is also related to its anti-inflammatory action and its regulation of the cellular redox state, driven by upregulation of the antioxidant enzymes and glutathione reduced (GSH), as well as by repression of two major pro-oxidant systems: NADPH oxidase and/or Cyp2E1 [41]. Nevertheless, the HGF/c-Met pathway in HCC contributes to tumor development by stimulating cell proliferation, invasion, and metastasis [40]. We observed that, in the cirrhotic liver induced by CCl_4, the hepatoprotector IFC-305 incremented HGF expression [38], which could have a protective role in the regenerative capacity of the liver. On the other hand, in DEN-induced HCC, the IFC305 treatment downregulated HGF and c-Met expression, which contribute to liver tumorigenesis reduction [39]. HGF and c-Met can be potentiated by ROS in hepatoma cells [41, 42]. It was described that, in the sequential model of cirrhosis-HCC with DEN, there are dysfunctional mitochondria and the administration of IFC-305 restored the mitochondrial function and regulated parameters implicated in metabolism, as well as the mitochondrial dynamics modified by DEN intoxication [43]. Therefore, the IFC-305 could be suppressing expression of HGF via the improvement of mitochondrial redox in DEN carcinogenesis. On the other hand, the restoration by IFC-305 treatment of the p53 protein expression in CCl_4-induced cirrhosis and in DEN-induced carcinogenesis, among other effects, could contribute to the restoration of ATP production by OXPHOS and to the decrease of ROS production. However, the exact molecular mechanism by which IFC-305 causes different effects on hepatocytes proliferation in cirrhosis and HCC requires further clarification.

3. Mitochondrial alterations in the HCC: the effect of the IFC-305 compound

Mitochondria are responsible for energy metabolism in eukaryotic cells; they generate ATP through oxidative phosphorylation. In addition, an important part of the ATP synthesis is the donation of electrons by the tricarboxylic acids chain (TCA) to the electron transport chain (ETC), constituted by five complexes (I-V), NADH enters complex I and generates NAD^+, and complex V forms ATP. Mitochondria regulate the energetic state, the redox state, and the metabolism of the cells, being able to generate the epigenetic intermediaries becoming the main therapeutic target of many kinds of cancer [44].

As a response to stress, the cells acquire a metabolic adaptation, which is an important area of research due to its relationship with different illnesses [45]. In chronic liver diseases like cirrhosis, energetic deficiency and alterations in energy parameters have been demonstrated independently of their etiology [46]. Otto Warburg suggested that mitochondria from tumor cells supply energy through glycolytic flow due to lack of oxygen or genetic-epigenetic alterations that affect oxidative metabolism [47]. Mitochondrial dysfunction is implicated in metabolic reprogramming in HCC. The increased ROS production and the reduced ATP generation may contribute to the HCC malignancy [48]. Metabolic alterations may decrease the

levels of acetyl CoA, which also plays an important role as modulator of gene expression [49]. In experimental models, including the CCl_4-induced cirrhosis, mitochondrial dysfunction has been demonstrated because impaired mitochondrial respiration and ATP decreased levels have been observed [50, 51]. A metabolic adaptation in response to the ATP diminished levels is increased glycolysis [51]. A consequence of oxidative stress in chronic liver diseases is the decrease in metabolic flux, which includes alterations in the TCA enzymes, such as isocitrate dehydrogenase (IDH), which can produce oncometabolites when it undergoes mutations [52].

The redox state can be represented by the NAD^+/NADH ratio, which is regulated by the ETC. Several enzymes depend on NAD^+ like sirtuin-1 (Sirt-1), a member of deacetylases, and poly (ADP-ribose) polymerase-1 (PARP-1). A Sirt-1 substrate is the peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α), which is upregulated in HCC and is responsible for orchestrating mitochondrial biogenesis, favoring accumulation of defective mitochondria [44]. On the other hand, PARP-1 modulates the transcription and DNA repair; however, in HCC, it is upregulated and is considered a hallmark of cancer [53]. The over-regulation of both enzymes in HCC may deplete the NAD^+ that can be related to loss of mitochondrial membrane potential (ψm) and mitochondrial dysfunction [54]. Alterations in ψm induce the process of mitochondrial dynamics as a repair response to possible damage to this organelle. Mitochondrial dynamics depends on two mechanisms: fission and fusion; the first one is caused by various types of stress and requires protein activity such as Drp-1, on the other hand, fusion requires the recovery of ψm and proteins such as mitofusin 1 and 2 (MFN 1 and 2) [44]. Mitochondrial fusion promotes cristae formation and normal mitochondria phenotype [55]. Morphological alterations in mitochondria determined through electronic microscopy in various models of hepatic fibrosis have been described a long time ago [56, 57].

Previously, it has been discussed some of the effects of adenosine (base molecule of IFC-305), which include increase in energy parameters and regulation of the redox state. Considering this background and what has been described regarding the metabolic and mitochondrial changes in chronic liver damage, such as cirrhosis and HCC, it was decided to evaluate whether IFC-305 had any mitochondrial effect in the sequential model of cirrhosis-HCC.

In the sequential model of cirrhosis-HCC, decreased mitochondrial respiration, determined through oxygen consumption, and a decreased ψm were found, which reflected in a diminished ATP synthesis. In fact, the dimeric form (active form) of the F1F0 complex of ATPase is lost [43].

On the other hand, alterations in the mitochondrial redox state were observed, determined through the ratio of the levels of β-hydroxybutyrate/acetoacetate (NAD^+/NADH). The activity of NAD-dependent enzymes was also affected, such is the case of IDH and PARP-1; this alteration induced a metabolic adaptation because increased levels of lactate were observed suggesting an increase in aerobic glycolysis [43].

It is known that the mitochondrion is capable of responding to several insults of stress through the activity of various nuclear-encoded proteins like PGC-1α and Sirt-1. However, the over-regulation of these proteins has been associated with the accumulation of dysfunctional mitochondria, as described above. In the model previously described, these proteins were found increased. Dysfunctional mitochondria have been related to their morphology,

and we know that morphology is closely linked to dynamism. The ratio of Drp-1/MFN-2, proteins that regulate the mitochondrial dynamics, was increased favoring the fragmented form of mitochondria as verified through electron microscopy [43].

Important findings were observed with the IFC-305 treatment as described in **Table 1** [43].

Uncoupled mitochondria depicted lower ATP synthesis due to the altered ψm and complex I activity. Previously, it has been demonstrated that complex I is sensitive to DEN toxicity, as NAD^+ linked respiration is inhibited [58]. Recovery of these parameters with IFC-305 treatment was observed, including the activity of NAD^+-dependent IDH. The PARP-1 activity inhibition probably favored the NAD^+ availability and contributed to the maintenance of the redox state. Mitochondrial function preservation and restoration allowed the normalization of the metabolism observed by lactate levels diminution.

On the other hand, the decreased Sirt-1 and PGC-1α in the groups treated with IFC-305 suggested that abnormal mitochondrial accumulation was inhibited. In fact, mitochondrial dynamics regulation was induced by IFC-305. These results demonstrated mitochondrial impairment through functional, metabolic, and dynamic alterations in HCC, and the hepatoprotector IFC-305 helps to repair them, being a tumor suppressive mechanism.

These findings support the mitochondrial role in the establishment of HCC and the interplay with the nuclear genome as targets in the design of new therapeutic strategies for the HCC treatment. In this regard, the IFC-305 supports that idea and emerges as a new possible HCC therapy through mitochondrial regulation.

According to the above, there is a growing interest to find pharmacological strategies to block the effects of mitochondrial dysfunction in HCC. Regarding this, in the model of HCC induced with DEN, a study was conducted to determine the mitochondrial effects of ginkgolide B in

Mitochondrial parameter	Effect
Function	Maintained and recovered:
	• mitochondrial respiration
	• ATP synthesis
	• mitochondrial membrane potential
	• dimeric form of the F1F0 ATPase subunit
	• normal cellular redox state
Metabolic	• Recovered the normal mitochondrial redox state
	• recovered the IDH activity
	• reduced lactate production
	• diminished increased PARP-1 activity
Dynamics	Avoided the accumulation of dysfunctional mitochondria through:
	• down-regulation of PGC-1α and Sirt-1
	• diminution of DRP-1/MFN-2 ratio
	• Sirt-3 increment

Table 1. Effects of IFC-305 administration in mitochondria in the sequential model of cirrhosis-HCC.

two different pharmaceutical formulations, finding a decrease in the mitochondrial generation of ROS and a decrease in the dissipation of the mitochondrial membrane potential [59]. Moreover, two of the most studied hepatoprotective compounds until now are resveratrol and N-acetylcysteine (NAC) [60]. On the one hand, resveratrol inhibits the formation of hepatocyte nodules in the DEN-induced HCC model plus phenobarbital administration; moreover, it is capable of modulating mitochondrial biogenesis [61]. On the other hand, NAC blocked phosphorylation of β-catenin, JNK, and c-jun activation, avoiding the development of liver damage in HCC transaldolase-deficient mice, a limiting enzyme for the non-oxidative branch of the pentose phosphate pathway, which is, at least in part, responsible for HCC generation [62]; furthermore, NAC stabilizes the mitochondrial membrane potential regulating mitochondrial dynamics [61].

4. Interaction of mitochondria and epigenetics in HCC: An overview

The epigenome can be altered not only by environmental factors, such as exposure to exogenous chemicals [63] but also by changes in the levels of endogenous cofactors and metabolites [64, 65]. The exact correlation between nucleus and mitochondrion allows for the maintenance of mitochondrial structure and function. On the one hand, the nuclear gene expression is regulated by mitochondrial intermediates, like acetyl-CoA, ATP, NAD^+, and s-adenosylmethionine, which are the link between the epigenome and calorie availability [47, 66]. In addition to the production of epigenetic substrates, mitochondria may be modified in their DNA (mtDNA). Some mitochondrial genes have been reported as hypermethylated in HCC; for example, mitochondrial ribosomal protein S12 (*Mrps12*), mitochondria-localized glutamic acid-rich protein (*Mgrap*), and transmembrane protein 70 (*Tmem70*) genes [67, 68]. On the other hand, the disruption of the step in the methylation of 5-mC to 5-hmC in the mitochondrial genome leads to the alteration of several OXPHOS genes, such as: NADH dehydrogenase (ubiquinone) 1 subunit C2 (NDUFC2), NADH dehydrogenase (ubiquinone) flavoprotein 1 (NDUFV1), NADH: ubiquinone oxidoreductase subunit S6 (NDUFS6) from complex 1. These modifications, added to the mitochondrial damage by oxidative stress, can favor the loss of ETC function. In addition to that, it has been reported that the mitochondrial genome damage can affect the expression of nuclear genes [69–71]. Moreover, there is a deregulation of hepatic one carbon, and TCA cycle, therefore it driving the aberrant epigenetics changes [72–74]. The main consequence of depressing the TCA cycle is the reduced availability of α-ketoglutarate, leading to a decrease in the activity of α-ketoglutarate-dependent proteins, which are responsible for the hydroxylation of many substrates in the cell that are important in epigenomic control [74].

Tumor cell metabolism can be linked to epigenetic changes during carcinogenesis; recent research has focused on epigenetic studies in relation to metabolic pathways [75, 76]. HCC is a heterogeneous disease affected by various lifestyles and environmental factors. Epigenetic alterations are frequently caused by these factors and contribute to hepatocarcinogenesis. During HCC development, different alterations in global DNA methylation have been described; for example, global hypomethylation leads to aberrant overexpression of oncogenes and large chromosomal instability [77, 78].

In cirrhosis and HCC, distinct patterns of aberrant DNA methylation associated with cirrhosis and HCC have been confirmed [79, 80].

5. Conclusion

The pathophysiology of HCC is multifactorial and involves mitochondrial dysfunction. Mitochondria usually generate relevant modulators of gene expression controlled by epigenetic mechanisms. These alterations induce chromosomic instability that could give advantages to subclones of cells to their outgrowth (**Figure 1**). Further studies are needed to find

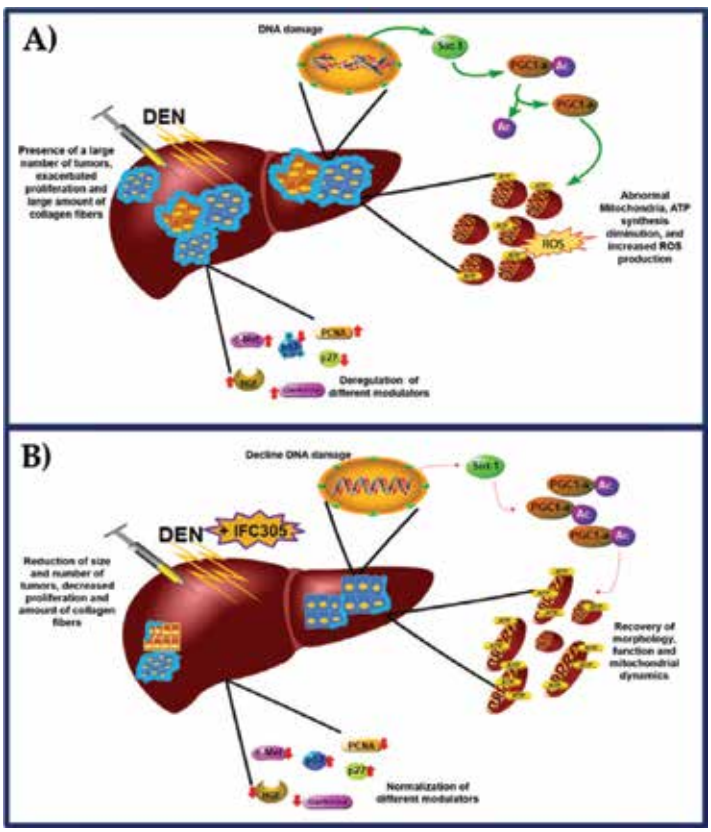

Figure 1. (**A)** In the model of liver injury induced by diethylnitrosamine (DEN), the architecture of the liver parenchyma is altered causing an exacerbated proliferation of various transformed clones, where the presence of a large number of tumors randomly distributed in each one is observed in the hepatic lobules. The preneoplastic nodules that form are surrounded by septa of collagen fibers; thus, favoring the evasion of the immune system and an ideal hypoxic microenvironment for the tumor cells. The genomic instability caused by the toxic as well as favoring mutations, for example in p53, and various alterations in different cellular modulators, among them HGF, c-Met, PCNA, gankyrin and p27. It also causes an increase of proteins, deacetylating PGC1-α, and, thus, modifies various nuclear genes exported to the mitochondria, causing accumulation of abnormal and dysfunctional mitochondria. (**B)** In the model of hepatocarcinoma induced by DEN, the administration of the adenosine derivative, IFC-305, has been shown to have various regulatory effects. The excessive accumulation of collagen fibers in preneoplastic nodules as well as the number and size of tumors are reduced. Also, cell morphology and DNA recover significantly. A decrease in the deacetylase Sirt-1, whose target is PCG1-α, has been observed, which allows the latter to remain acetylated and can be internalized to mitochondria, where it will promote its adequate morphology, dynamics and function. It has also been found that the compound IFC-305 acts on the levels of some important modulators in cancer (p53, HGF, C-Met…), maintaining or returning them to their concentrations under normal conditions. Overall, the aforementioned effects make this compound a possible therapeutic alternative.

therapeutic strategies capable of maintaining and improving the mitochondrial integrity to avoid alterations in the epigenetic regulation of nuclear- and mitochondrial-encoded genes. These effects could suppress failures in cell cycle checkpoints and the uncontrolled proliferation to prevent or reverse HCC as demonstrated for IFC-305.

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Conflict of interest

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Abbreviations

HCC	hepatocellular carcinoma
PARP-1	poly (ADP-ribose) polymerase-1
TACE	transcatheter arterial chemoembolization
HSC	hepatic stellate cells
IL-1	interleukin-1
IL-6	interleukin-6
ECM	extracellular matrix
HGF	hepatocyte growth factor
IGR	insulin growth factor
TGF-β	transforming growth factor-β
MMPs	metalloproteinases
NAD+	nicotinamide adenine dinucleotide oxidized
NADH	nicotinamide adenine dinucleotide reduced
CCl4	carbon tetrachloride
IFC-305	aspartate of adenosine

VEGF	Vascular Endothelial Growth Factor
OXPHOS	oxidative phosphorylation
SCO2	chaperone protein "synthesis of cytochrome c oxidase 2"
ROS	reactive oxygen species
TIGAR	TP53-induced glycolysis and apoptosis regulator
PCNA	proliferating cell nuclear antigen
DEN	diethylnitrosamine
GSH	glutathione reduced
TCA	tricarboxylic acids chain
ETC	electron transport chain
IDH	isocitrate dehydrogenase
Sirt-1	sirtuin-1
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
ψm	mitochondrial membrane potential
MFN 1	mitofusin 1
MFN 2	mitofusin 2
NAC	N-acetylcysteine
mtDNA	mitochondrial DNA
Mrps12	mitochondrial ribosomal protein S12 gene
Mgrap	mitochondria-localized glutamic acid-rich protein gene
Tmem70	transmembrane protein 70 gene
NDUFC2	NADH dehydrogenase (ubiquinone) 1 subunit C2
NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1
NDUFS6	NADH: ubiquinone oxidoreductase subunit S6

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Chapter 9

Biologic and Immunotherapy Developments in Advanced Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, and the second leading cause of cancer-related mortality worldwide with a very poor 5-year survival. Treatment for HCC includes surgery, liver-directed therapies and systemic therapies. Until 2008, no effective systemic therapy was available for advanced HCC. Sorafenib is the first drug to show improvement in overall survival among patients with advanced HCC in comparison to placebo, and it is approved by U.S. Food and Drug Administration (FDA) as a first-line treatment of advanced HCC. After sorafenib approval, several targeted and immune therapies were tested and showed efficacy in advanced HCC. Lenvatinib has been shown to be non-inferior to sorafenib as first-line treatment. Both nivolumab and regorafenib showed improvement in overall survival among patients with advanced HCC as a second line treatment after progression on sorafenib, and both are FDA approved for this indication. There is a limited role for cytotoxic agents in the treatment of advanced HCC.

Keywords: hepatocellular, carcinoma, HCC, kinase, inhibitors, TKI, VEGFR, sorafenib, lenvatinib, regorafenib, immunotherapy, PD-L1, nivolumab

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver. HCC is the second leading cause of cancer-related mortality worldwide with a very poor 5-year survival. The incidence of HCC has been increasing over the past decades [1]. Risk factors for HCC include hepatitis B and C infection, alcohol use, non-alcoholic steatohepatitis, and aflatoxin.

Treatment approaches for HCC depend on the stage and the hepatic function, and includes surgical therapies (liver transplantation, resection, and ablation) and nonsurgical therapies, which may be liver-directed (percutaneous ethanol injection, radiofrequency ablation, transarterial embolization, external beam radiation therapy) or systemic therapies.

Until 2008, there was no effective systemic therapy for advanced HCC. Cytotoxic chemotherapy has not been used routinely as of low efficacy and poor functional status for patients with advanced HCC, who often have cirrhosis. Since the advent of sorafenib in 2008, there has been a surge of several targeted and immune therapies with various degree of effectiveness. In this chapter, systemic therapies for advanced HCC will be reviewed. Those include oral kinase inhibitors, antiangiogenic monoclonal antibodies, immune-therapeutic approaches and cytotoxic chemotherapies.

2. Kinase inhibitors

2.1. Sorafenib

Sorafenib is a multi-kinase inhibitor of vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptor (PDGFR) and Raf family kinases [2]. Sorafenib has shown to improve overall survival in comparison to placebo in advanced HCC, and it was the first drug to get Food and Drug Administration (FDA) approval as a first-line treatment for Child-Pugh score-A HCC. In the multicenter European SHARP trial, 602 inoperable HCC and Child-Turcotte-Pugh-A cirrhosis patients, were assigned to sorafenib (400 mg twice daily) or placebo [3]. The primary endpoint of the trial was overall survival, which was significantly longer in the sorafenib-treated group (10.7 vs. 7.9 months). Time to radiologic progression was also longer (5.5 vs. 2.8 months). Objective response rates were low at 2%.

Sorafenib was well tolerated in this trial. Diarrhea and hand-foot skin reaction were the only grade 3 or 4 adverse effects that occurred significantly more often in the treated group; (8 vs. 2%) and (8 vs. <1%) respectively. There were no differences in liver dysfunction or bleeding.

An exploratory analysis of SHARP trial showed that hepatitis C related HCC has the highest median overall survival advantage of 6.6 months (14 vs. 7.4 months). This is in comparison to 3.6 months (9.7 vs. 6.1 months) in those with HBV related cirrhosis and 2.3 months (10.3 vs. 8 months) in those with alcohol-related liver disease [4].

Hepatitis B virus is more prevalent in the Asian patients than in the Western population. Sorafenib was tested as a first-line treatment in Asian patients in a placebo-controlled phase III trial in which 226 patients with Child-Turcotte-Pugh A cirrhosis received sorafenib 400 mg twice daily or placebo [5]. Patients receiving sorafenib had significantly higher median overall survival (6.5 vs. 4.2 months). Grade 3 or 4 side effects were similar to SHARP trial.

Based on the results of SHARP trial, the FDA approved sorafenib monotherapy as first-line therapy for unresectable HCC.

It is worth mentioning that the patients enrolled in the above trials had mostly Child-Turcotte-Pugh A cirrhosis. This is not representative of the real practice where a significant number of

patients have more advanced cirrhosis. FDA approval of sorafenib for HCC did not particularly specify the underlying cirrhosis state. Data regarding safety and efficacy of sorafenib in patients with Child-Turcotte-Pugh B or C cirrhosis are limited, and suggest that patients have poorer overall survival and overall worse side effect profile in comparison to patients with Child-Turcotte-Pugh A. Advanced progressive cirrhosis rather than sorafenib itself might be an explanation for such differences [6, 7].

Sorafenib is associated with several side effects such as hypertension, cardiotoxicity, arterial thromboembolism, bleeding, renal toxicity, hand-foot skin reaction and others. Sorafenib has been associated with potentially fatal liver toxicity. Liver function tests should regularly be monitored during treatment.

2.1.1. Combining sorafenib with doxorubicin

In a phase II trial, the combination of six cycles of doxorubicin with sorafenib 400 mg twice daily was compared to sorafenib and placebo [8]. Combination therapy was associated significantly longer median time to tumor progression (6.4 vs. 2.8 months) and median overall survival duration (13.7 vs. 6.5 months). The side effect profile was not significantly worse with combined therapy. However those results were not reproduced in the randomized phase III trial, Cancer and Leukemia Group B [CALGB] trial 80,802 [9]. The study was stopped early by the data monitoring safety board after a planned interim analysis suggested futility for the combination. In a preliminary report presented at the 2016 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium, median overall survival was not significantly better for the combination (9.3 vs. 10.5 months), nor was median progression-free survival (3.6 vs. 3.2 months), but toxicity was worse.

2.2. Lenvatinib

Lenvatinib is a multi-kinase inhibitor of VEGFRs, fibroblast growth factor receptors (FGFR), (PDGFR), c-Kit, and the RET proto-oncogene [10].

A randomized noninferiority trial, the REFLECT study, compared lenvatinib (12 mg once daily for body weight ≥60 kg, 8 mg daily for <60 kg) with sorafenib (400 mg daily for all patients) in 954 patients with unresectable HCC and no prior systemic therapy (99% Child-Turcotte-Pugh A) [11]. The predefined noninferiority margin (primary endpoint overall survival) was 1.08. Lenvatinib was noninferior to sorafenib (median overall survival 13.6 vs. 12.3 months, hazard ratio 0.92, 95% CI 0.79–1.06), the objective response rate was higher (24 vs. 9%), and median time to progression was longer (7.4 vs. 3.7 months, hazard ratio 0.66, 95% CI 0.57–0.77). Lenvatinib leads to higher grade 3 or 4 hypertension (23 vs. 14%), while sorafenib was associated with higher hand-foot skin reaction (11 vs. 3%).

Lenvatinib was approved in Japan in March 2018 for unresectable HCC. Lenvatinib is not approved by FDA yet.

Both sorafenib and lenvatinib can be used in the first-line treatment of advanced HCC. There are no data on second-line treatment after lenvatinib and whether lenvatinib is effective as a second line after sorafenib.

2.3. Regorafenib

Regorafenib is an antiangiogenic (including VEGFR-1, VEGFR-2, and VEGFR-3), anti-stromal, and an oncogenic tyrosine kinase inhibitor that is structurally similar to sorafenib [12].

In the randomized RESORCE trial, 573 patients who received sorafenib for at least 20 days at a dose of at least 400 mg daily and who had radiologic progression were randomly assigned to regorafenib (160 mg once daily for 3 weeks on and 1 week off) or placebo [13]. Regorafenib was associated with significantly higher median OS (10.6 vs. 7.8 months, hazard ratio for death 0.63) and disease control (objective response plus stable disease; 65 vs. 36%).

Treatment was relatively well tolerated; grade 3 or 4 hypertension, hand-foot skin disease and fatigue were more frequent with regorafenib. Sixty-eight percent of patients treated with regorafenib required dose modification for adverse events compared with 31% of the placebo group.

In April 2017, the FDA expanded the indications for regorafenib to include patients with HCC who had been previously treated with sorafenib.

Regorafenib is an alternative to nivolumab for second-line HCC treatment. There are no trials comparing regorafenib with nivolumab in this setting.

2.4. Cabozantinib

Cabozantinib is another inhibitor of several receptor tyrosine kinases, including the hepatocyte growth factor/c-MET and VEGFR [14]. Efficacy in patients with previously treated advanced HCC was shown in the placebo-controlled phase III CELESTIAL trial [15]. In a preliminary report, in the group of patients receiving second- or third-line treatment, median overall survival was significantly better with cabozantinib (10.2 vs. 8.0 months), and the difference was more pronounced when the analysis was limited to patients whose only prior therapy was sorafenib (median overall survival 11.3 vs. 7.2 months). The most common grade 3 or 4 adverse events with cabozantinib were palmar-plantar erythrodysesthesia (17 vs. 0 in the placebo group), hypertension (16 vs. 0%), increased aspartate aminotransferase (12 vs. 7%), fatigue (10 vs. 4%), and diarrhea (10 vs. 2%).

2.5. Axitinib

Axitinib is a selective kinase inhibitor that inhibits VEGFR. Axitinib was not superior to best supportive care alone in a randomized phase II trial comparing best supportive care plus axitinib (starting dose 5 mg twice daily) with placebo in 202 patients with advanced HCC who progressed on or were intolerant of one prior antiangiogenic therapy [16]. The difference in median overall survival (the primary endpoint), was not statistically significant (12.7 vs. 9.7).

2.6. Sunitinib

Sunitinib is another orally active multi-kinase inhibitor that targets a variety of tyrosine kinases in addition to VEGFR, including PDGFRs, KIT, RET, and FMS-like tyrosine kinase 3 (FLT3) [17].

Sunitinib was significantly inferior to sorafenib in a phase III trial that directly compared both drugs in 1073 previously untreated patients with advanced HCC [18]. The trial was closed prematurely when an interim analysis revealed that patients receiving sunitinib had significantly worse survival (median 7.9 vs. 10.2 months) and more frequent and severe treatment-related toxicity.

3. Antiangiogenic monoclonal antibodies

3.1. Bevacizumab

Bevacizumab is a monoclonal antibody directed against VEGFR that has some activity in advanced HCC. Efficacy was shown in a trial in which 46 patients with advanced nonmetastatic HCC received single-agent bevacizumab at a dose of either 5 or 10 mg/kg once every other week [19]. An objective response was documented in six (13%, one complete), and the median progression-free survival was 6.9 months. The most common grade 3 or 4 toxicities were hypertension (15%), thrombosis (6%), and major bleeding (11%).

Bevacizumab is also active in combination with capecitabine, with or without oxaliplatin [20, 21], and gemcitabine combined with oxaliplatin (GEMOX) [22]. Whether any of those combination regimens are better than bevacizumab alone is not clear and will require randomized trials.

3.2. Ramucirumab

Ramucirumab is a recombinant monoclonal antibody that binds to VEGFR-2. The REACH trial failed to show a significant survival advantage relative to placebo (median overall survival 9.2 vs. 7.6 months) in patients with advanced HCC who progressed on sorafenib [23]. An unplanned group analysis suggested a possible survival benefit in patients with a high initial level of alpha-fetoprotein (AFP) above 400 ng/mL) at diagnosis. A follow-up phase III trial in patients with AFP-elevated HCC is ongoing.

4. Immunotherapeutic approaches

4.1. Introduction

Immune-based approaches that focus on vaccination strategies, cytokines or non-specific T cell activation have been tested for many years in HCC without promising result. However, the recent advancement in immune-oncology with the FDA approval of many immune checkpoint inhibitors, sparked a great interest in the immune-based treatment approaches for patients with HCC. The strategy of adopting an immunocentric approach to HCC treatment may be potentially more efficacious and less toxic. Interestingly, what makes the immunotherapy appealing in liver cancer is that HCC is a high immunogenic cancer, due to high blood flow with unique vast tumor antigen repertoire because of mutations and aberrant expression profiles [24]. On the other hand, there is an inherently immunosuppressive microenvironment

of the liver; "Tolerogenic Liver"; that helps evade the immune response. The liver's pathway to immune tolerance is multifactorial. T-cells are stimulated through a dual signaling pathway that requires the interaction of T cell receptors (TCR) with major histocompatibility complex (MHC)/peptide complexes on antigen presenting cells (APCs) and expression of co-stimulatory molecules on T cells and APCs. Down-regulation of MHC class I molecules on tumor cells induces impairment of tumor antigen processing and presentation [25]. In addition, a reduced expression of co-stimulatory molecules, such as B7-1 and B7-2, in HCC leads to T cell anergy [26]. Programmed cell death protein-1 (PD-1) overexpression in tumors promotes immune evasion and tumor growth by suppressing T-cell response [27]. PD-L1 is not the only immunosuppressive factor in the tumor microenvironment. HCC immune evasion can also be achieved through overexpression of MHC class II molecules in tumor cells, which leads to $CD4^+$ T cell anergy in the absence of co-stimulatory molecules (CMs) on T cells and APCs. A better understanding of the antigenic profile of HCC and tumor microenvironment has helped to develop a refined immunotherapeutic strategies in treatment of HCC [28].

4.2. Indirect immunological strategies

4.2.1. Checkpoint inhibitors

Checkpoint Inhibitors play critical roles in cancer immunology. Blockading the PD-1/PD-L1 pathway could modulate the tumor microenvironment, reactive T-cell and prime the endogenous antitumor immune responses. Treatment with checkpoints inhibitors have shown benefits in clinical trials of HCC. Common immune checkpoint proteins include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), PD-1, programmed cell death ligand one (PD-L1), lymphocyte activation gene three protein (LAG-3), B and T lymphocyte attenuator (BTLA), T-cell immunoglobulin and mucin-domain-containing (TIM-3), VISTA and OX40 [29, 30].

4.2.1.1. CTLA-4 inhibitors

CTLA-4 is constitutively expressed in activated T cells and NK cells [31]. CTLA-4 inhibitors prevent the binding of CTLA-4 to B7-1 and B7-2, thereby actively encourage the activation of T cells. CTLA-4 was the first checkpoint studied in HCC. Tremelimumab, an anti-CTLA-4 monoclonal antibody, was tested in a phase II study in a 21 patients with advanced HCC and hepatitis C. The disease control rate was (76.4%), median OS and PFS were 7.5 and 6.4 months respectively. Moreover, viral loads of HCC were significantly reduced. Although a short-lived remarkable rise in serum transaminases was observed after the first dose, no patients experienced immune-related adverse events or serious hepatotoxicity [32]. In another non-comparative clinical trial involving patients with advanced HCC, a combination therapy of tremelimumab and radiofrequency ablation increased the number of intratumoral $CD8^+$ T cells and reduced HCV viral loads [33].

4.2.1.2. PD-1 inhibitors

The PD-L1/PD-1 pathway is another mechanism of tumor-induced immune tolerance. PD-1 expression on effecter phase $CD8^+$ T cells is increased in patients with HCC compared to

no HCC cirrhotic patients [34]. Moreover, there is frequent and early disease progression in patients with HCC with higher numbers of tumor-infiltrating and circulating PD-1 + $CD8^+$ T cells post hepatic resection [35]. Therefore, a supporting great rationale exists for using PD-1 and PD-L-1 blocking antibodies against HCC. Some PD-1 inhibitors, such as nivolumab, pembrolizumab, and pidilizumab, have been investigated for cancer treatment.

The CheckMate-040 phase I/II trial studied the safety and antitumor effect of nivolumab in 48 patients with advanced HCC [36]. The target population included patients with intermediate or advanced HCC and preserved liver function (Child-Turcotte-Pugh-A cirrhosis) who were candidates for systemic therapy and had progressed or were intolerant to sorafenib. In the escalation and expansion cohorts, objective tumor responses occurred in 15 and 20% of patients, respectively. There were durable responses that lasted for a median of 17 months. An additional 45% of patients had a stable disease associated with durability, lasting 6 months at minimum. Those responses were consistent across the different HCC risks, and both in sorafenib-naïve and sorafenib-exposed patients.

Overall, frequencies of grade 3/4 treatment-related AEs were 20%. Only 3% of patients discontinued nivolumab because of treatment-related adverse events, while no treatment-related deaths occurred. Elevated transaminases was the most frequent laboratory alteration (20%). However, only 5% of the patients had grade 3 or higher. Immune-related hepatitis requiring steroid therapy. CheckMate-040 showed that nivolumab might be effective with acceptable toxicity in HCC, regardless of hepatitis status. On September 22, 2017, and based on the outcome of CheckMate-040 study, the FDA granted accelerated approval to nivolumab for the treatment of hepatocellular carcinoma (HCC) as second-line therapy in patients who have been previously treated with sorafenib.

CheckMate-459 is an ongoing phase III study, (NCT02576509) that randomizes patients with advanced HCC to either nivolumab or sorafenib in the first-line setting [37].

The efficacy and safety of pembrolizumab in HCC has been investigated. The phase I/II study KEYNOTE-224 tested pembrolizumab in 104 patients with advanced HCC who progressed on sorafenib. The overall response rate was 16.3%. Durable response was seen with 94% of responders were estimated to have a response duration of 6 months or longer. The median PFS was 4.8 months, and the median OS was not been reached. The safety profile was generally comparable to that established for pembrolizumab monotherapy in other indications, and no viral flares were seen [38].

4.2.2. Oncolytic immunotherapy

Targeting tumor vasculature by oncolytic viruses (OVs) is an attractive strategy that offers several advantages. Oncolytic viruses are wild-type or engineered viruses that selectively target and replicate in cancer cells and cause lysis without harming normal tissues [39]. The underlying mechanism of the antitumor activity for oncolytic viruses involves direct killing of tumor cells by expanding in the cells and causing cell lysis. Different from normal cell, viruses can expand in cancer cells considerably due to the impairment of the tumor's defense mechanisms against viral infection. [40–43]. In addition,, OVs can initiate antitumor immune response by

triggering key signals through oncolysis to dendritic cells (DCs) and other antigen-presenting cells (APCs) [44]. OVs have some advantages over other treatment modalities, those include: the low probability of generating resistance as OVs often target multiple oncogenic pathways; OVs replicate in a tumor-selective fashion with minimal systemic toxicities; and virus dose in the tumor increases over time due to in situ virus amplification, as opposed to classical drug pharmacokinetics that decreases with time [45]. The efficacy of an evolutionary cancer-favoring engineered vaccinia virus (CVV) was investigated in an animal model of metastatic HCC. In this animal study, the subjects were randomized into sorafenib, CVV, or sorafenib with CVV. Metastatic regions were interestingly rare in the CVV-treated groups (i.e., CVV or sorafenib with CVV) whereas metastatic regions existed in the sorafenib-treated group [46].

JX-594 is a thymidine kinase gene-inactivated oncolytic vaccinia virus engineered for the expression of transgenes encoding human granulocyte-macrophage-colony-stimulating factor (GM-CSF) and β-galactosidase, which increases antitumor immune responses [39, 47–49] This virus is safe in humans and extremely toxic to cancer cells.

Oncolytic viruses have produced enough therapeutic efficacy with great optimism in the future trials. Although the initial concerns of clinical investigators were for safety like a risk of viral infection or introduce oncogenic mutation, these have proven not to be a significant issue in these trials.

4.2.3. HCC vaccines

Cancer vaccination is performed by utilizing antigenic substances to stimulate tumor-specific immune responses that can remove cancer cells and prevent recurrences. HCC vaccines include cancer cells, antigen peptides, DCs, and DNA-based.

4.2.3.1. Antigen peptide vaccines

Peptide-based tumor-associated antigens (TAAs), such as alpha-fetoprotein (AFP), GPC3, SSX-2, NY-ESO-1, human telomerase reverse transcriptase (hTERT), HCA587, and melanoma antigen gene-A (MAGE-A), are excellent vaccine targets for the treatment of HCC [50].

AFP which normally originates from embryonic liver cells, can be overexpressed on HCC cell surfaces. However, immune responses to AFP are limited due to acquired immune tolerance during the development of the immune system. To overcome this tolerance, a research group investigated the use of a recombinant rat AFP to induce cross-reactions between xenografts and endogenous molecules in animals and observed modest cellular and humoral immune responses [51]. In a phase II trial of GPC3-derived peptide vaccine for HCC, 25 patients received 10 vaccinations over 1 year after surgery. The recurrence rate in patients who underwent both surgery and vaccination was significantly lower than the rate in 21 patients who underwent surgery only (24% vs. 48 and 52.4% vs. 61.9% at 1 and 2 years, $p = 0\ 047$ and 0.387, respectively), demonstrating the efficacy of the GPC3-derived vaccine [52].

4.2.3.2. Dendritic cell (DC) vaccines

DCs, were found to be the most powerful APCs in the body's immune system, and capable of stimulating naïve T cells and driving primary immune responses. A phase I/IIa comparative

study with 30 patients with advanced HCC stratified into mature autologous DCs pulsed, and other control group received supportive treatment. The result demonstrated an improvement in overall survival with two patients (13.3%) partial radiological response was observed, and nine patients (60%) has stable disease. The study concludes using tumor antigen-pulsed DCs vaccine can be effective adjuvant therapy with other treatment modalities of HCC or palliative treatment option in advanced HCC where other treatment options are not applicable [53]. In addition, the safety and tolerance of DC vaccines have been confirmed in patients with HCC [54].

4.3. Direct immunological strategy

4.3.1. Adoptive cell therapy

Adoptive cell therapy (ACT) is an immunotherapeutic approach that attacks cancer cells using genetically engineered patients' lymphocytes. It functions by stimulating or loading autologous lymphocytes with cytokines or tumor antigens, cultivating them ex vivo and then re-infusing them into the patient [55–57]. Adoptive immunotherapy for HCC includes cytokine-induced killer (CIK) cells, tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, and chimeric antigen receptor (CAR) T cells. The effectiveness and safety of ACT in patients with HCC have been studied in many experiments, which paved the road for its clinical implication.

4.3.1.1. Cytokine induced killer cells (CIK)

CIK cells are a heterogeneous MHC independent cell population which are able to both recognize tumor antigens and kill cancer cells directly [58, 59]. In a phase III study of adjuvant CIK therapy after radical resection for HCC, patients were randomized to receive four cycles of CIK therapy or no treatment. The median time to recurrence (TTR) was 13.6 months in the CIK group and 7.8 months in the control group ($p = 0.01$), All adverse events were grade 1 or 2. There were no significant differences in incidence between the two groups, indicating the safety and efficacy with respect to prolonging TTR of CIK therapy in patients with HCC. However, there were no statistically significant differences between the groups in disease-free survival (DFS) and overall survival (OS) [60]. In addition, a meta-analysis of 693 patients with HCC demonstrated that a combination of dendritic cell- (DC-) CIK cells and TACE improves 1-and 2-year OS, overall response rate (ORR), disease control rate (DCR), and the quality of life [61].

4.3.1.2. Tumor infiltrating lymphocytes (TILs)

TILs are autologous tumor-infiltrating lymphocytes (TIL), which are derived from tumor tissues and are cultured and induced using IL-2 and anti-CD3 antibodies ex vivo [62–64]. Reinfusion of autologous TILs, which possess tumor-specific immunity, may target multiple tumor antigens. Low toxicity of autologous TILs was verified in a phase I study involving patients with HCC, suggesting a novel treatment option [65]. To date, TILs have not been well characterized, mainly due to difficulties in purifying and expanding them.

4.3.1.3. Natural killer cells (NKCs)

NK cells are component of innate immune system and can directly kill tumor cells and infected cells without preliminary sensitization or MHC restriction [66]. However, they lack

the ability to target tumor cells and can injure normal liver tissues. In a previous series of experiments, the cytotoxicity of NK cells against HCC cells was enhanced by first generating a new hepatoma cell line, K562-mb15-41BBL, which achieved a more efficient stimulation of NK cells in vitro [67]. Furthermore, HCC cells exposed to 5 μmol/L sorafenib for 48 h showed high sensitivity to NK cells. Finally, NKG2D, an engineered NK-cell-activating receptor, was tested in vitro and in mice. All of the outcomes were positive in increasing the cytotoxicity of NK cells, providing the possibility of further clinical trials in HCC.

4.3.1.4. CAR-T cell

Chimeric antigen receptor redirected-T cells (CAR-T cells) are genetically modified T lymphocytes that specifically target tumor-associated antigens (TAAs) and kill cancer cells in an MHC-independent manner [68, 69]. CAR-T cells have achieved inspiring outcomes in patients with B cell malignancies with great therapeutic efficacy in leukemia and lymphoma therapy. CAR T therapy is being studied for solid tumors, such as HCC [70]. In some solid tumors with a tremendous phenotypic heterogeneity, CAR T cells could target the tumor antigen and cause antigen-positive cell death, while antigen-negative cancer cells may induce tumor relapse. However, Cart T cell structure engineering has been evolved significantly. Recently, CAR T cells with a transgenic "payload or TRUCK," also called the "fourth generation" CAR T cells, were designed [71]. This CAR T cells work by releasing inducible cytokines such as IL-12 which will augment T cell activation and further activate innate immune system to kill antigen negative cancer cells. Specific Tumor-associated antigens in HCC that recognized by cytotoxic T lymphocytes (CTLs) have been investigated. GPC3, which usually correlates with poor prognosis in HCC, has been demonstrated as a promising liver cancer-specific target in multiple studies, due to its overexpression in HCC and limited expression in normal tissues [72] GPC3-targeted CAR T cells could providing promising therapeutic intervention for GPC3-positive HCC. The ability of GPC3-targeted CAR T cells to eliminate GPC3-positive HCC cells was confirmed both in vivo and in vitro, and the survival of mice with HCC xenografts was prolonged with CAR T cell therapy in vivo [73]. In another study, T cells with two complementary CARs against GPC3 and asialoglycoprotein receptor 1 (ASGR1) decreased the risk of on-target, off tumor toxicities and demonstrated potent antitumor immune responses targeting $GPC3^{+}$ $ASGR1^{+}$ HCCs both in vivo and in vitro [74]. However, to date, the related studies conducted have been predominantly basic, and more clinical trials are required to prove the efficacy of CAR T in HCC.

4.4. Combination strategies

Combination therapies include combinations of different checkpoint inhibitors with TKIs, oncolytic viruses, small molecules and ablative therapies.

Combining anti-PD-1 with sorafenib has been studied in an animal model in HCC. The results showed efficacy only with the concomitant targeting of the hypoxic and immunosuppressive microenvironment with agents such as CXCR4 inhibitors, and not when combined with sorafenib alone [75]. According to these results, a potential future approach could be by careful titration of VEGF inhibition with the aim to block the VEGF pathway and contemporarily alleviate hypoxia by vascular normalization, enhancing immunotherapy efficacy [76].

Checkpoint inhibitors combinations have also been studied, as a way to improve synergy and overcome resistance. PD-L1 is not the only immunosuppressive factor in the tumor microenvironment. The regulatory T cells (Treg) stands out among the immunosuppressive cells of the tumor microenvironment. Anti-CTLA-4 agents deplete tumor-associated Treg via an FccR dependant mechanism in preclinical models and have promising result in malignant melanoma [77].

5. Cytotoxic chemotherapies

Historically, traditional chemotherapeutic agents have not shown great efficacy in the treatment of HCC when used in the advanced disease stage, in particular in case of progression after locoregional therapy. Moreover, conventional cytotoxic chemotherapies have not provided a clinical benefit or prolonged survival for patients with advanced HCC. There are limited data supporting the use of cytotoxic chemotherapies in unresectable disease, and it should be used preferably in the context of a clinical trial [78].

6. Conclusions

Advanced HCC remains a deadly disease with limited systemic treatment options. The advent of sorafenib as first-line treatment ignited a plethora of trials testing various targeted and immunotherapeutic approaches. Currently, both regorafenib and nivolumab are FDA approved for second-line treatment among patients with advanced HCC who progressed

Agent	Type	MOA	FDA Approved	Line of treatment	Trial	Positive outcome	Ref.
Sorafenib	MKI	VEGFRs, PDGFR, TKI	Yes	First line	SHARP	OS, PFS	[3]
Lenvatinib	MKI	VEGFR, FGFR, PDGFRs, c-kit	No	First line	REFLECT	Non-inferior to sorafenib	[11]
Regorafenib	MKI	VEGFR, anti-stromal TKI	Yes	Second line	RESORCE	OS, DCR	[13]
Cabozantinib	MKI	VEGFR, MET, AXL c-KIT	No	Second or third line	CELESTIAL	OS	[15]
Nivolumab	IgG4 McA	Anti-PD-1	Yes	Second line	CheckMate-040	DCR, OS,PFS	[36]

List of abbreviations: MOA: mechanism of action, Ref: references, DCR: disease control rate, FGFR: fibroblast growth factor receptor, McA: monoclonal antibody, MKI: multi-tyrosine kinase inhibitor, OS: overall survival, PFS: progression free survival, PDGFR: platelet-derived growth factor receptor, PD-1: programmed death-1, TKI: trosine kinase inhibitor, VEGFRs: vascular endothelial growth factor receptors.

Table 1. Most common systemic agents for advanced HCC.

on sorafenib. The list of available treatment options (**Table 1**) is expected to increase with the encouraging results of several ongoing early phase trials, which eventually will lead to improvement in patients survivals.

Conflict of interest

Anwaar Saeed, MD declares research grants from AstraZeneca and Exelixis.

The remaining authors declare that they have no conflict of interests.

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This book offers remarkable coverage of liver cancer from etiology to prevention and treatment. It provides an updated and new vision of this major cancer that continues to affect hundreds of thousands of people and remains one of the leading causes of cancer deaths around the world. To ensure the high quality of this book, important insights are included and rigorously discussed in a simple and authentic way. The book includes detailed and updated descriptions of the main causes of liver cancer and also the prevention and treatment of this disease. This book is a relevant source of knowledge, very useful for researchers, medical doctors, medical residents, students, healthcare providers, public health decision makers, and all individuals interested in the prevention of this disease.

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