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Hepatocellular Carcinoma: Diagnosis, Therapy and Molecular Investigations

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Abstract

Hepatocellular carcinoma (HCC) is the leading cause of primary liver cancers. Surveillance of individuals at specific risk of developing HCC, early diagnostic markers, and new therapeutic approaches are essential to obtain a reduction in disease-related mortality. In the last decades imaging technologies, statistical models, and standardized diagnostic procedures associated with clinical guidelines for intervention significantly enhanced the success rate and life expectancy of HCC patients. In addition, the work of several research laboratories contributed to the identification of markers and pathways altered in HCC which represent potential biomarkers or molecular targets for therapy. In this short review we describe the current approaches for HCC diagnosis and treatment and the most recent advancements in the characterization of biomolecular mechanisms implicated in the pathogenesis and progression of HCC.

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancers and, with rapidly increasing incidence in the last two decades, constitutes a major global health problem [1,2]. Its pathogenesis is based on multifactorial mechanisms of chronic liver injury and inflammation, such as HBV/HCV infection, alcohol abuse, non-alcoholic steatohepatitis (NASH), aflatoxin intoxication, or congenital metabolic disorders [1,2]. As a result, up to 90% of HCC develops in the context of liver cirrhosis, which further complicates the therapeutic management of these patients due to the inherent risk of post-treatment liver failure [1,2]. In recent years, the prognosis of HCC patients has progressively improved thanks to multimodal interventions in prevention, diagnosis, and therapy [1,2]. HBV vaccination campaigns and the implementation in clinical practice of direct acting agents (DAA) against HCV have effectively controlled the most impactful oncogenic triggers for HCC.

Statistical models have also been developed to predict the probability of HCC occurrence in patients with chronic liver diseases [3]. In 2014, Johnson et al. proposed a serum biomarker-based statistical model for estimating the likelihood of HCC in these patients. The GALAD model considers serum biomarkers such as AFP, AFP-L3, and des-γ-carboxyprothrombin (DCP) in combination with gender and age [4,5]. Its accuracy becomes even more specific when an AFP glycoform specific for malignant tumors and HCC inflammation and a prothrombin precursor, which makes it possible to distinguish HCC from non-malignant liver diseases, are also analysed.

Imaging technology advances have enhanced the diagnostic power of multiphasic computed tomography (CT) scan, dynamic contrast-enhanced magnetic resonance imaging (MRI), and contrast-enhanced ultrasound (CEUS) scan [1,2,6]. The American College of Radiology has developed the Liver Imaging Reporting and Data System (LI-RADS), which is a comprehensive system for standardizing the acquisition, interpretation, reporting, and data collection of liver imaging [7]. According to LI-RADS, diagnostic hallmarks of HCC include arterial phase hyperenhancement, non-peripheral venous or delayed phase washout appearance, enhancing capsule appearance,

and threshold growth. Importantly, imaging criteria apply only to patients with cirrhosis or current/prior HCC, and for nodules >1 cm. In these cases, the prevalence of HCC is sufficiently high and the diagnostic performance of imaging sufficiently precise that lesions meeting imaging criteria for HCC have close to 100% probability of being HCC [7]. Once the diagnosis is established, prognostic assessment is a critical step in the clinical decision making of HCC management, evaluating not only tumor stage but also liver function and performance status.

The current European Association for the Study of the Liver Clinical Practice Guidelines endorse the Barcelona Clínic Liver Cancer (BCLC) staging system [1]. Hepatic resection may be a potentially curative therapy for patients with good performance status, preserved liver function, and solitary tumor of any size with no evidence of gross vascular invasion. In these cases, liver resection is associated with a 5-year survival rate of about 70% [1]. However, tumor recurrence can be observed in 50–70% of cases within 5 years following surgery, either as intrahepatic metastases (often within 2 years following surgery) or as new HCC in the remaining cirrhotic liver (occurring more often beyond 2 years) [1]. When the severity of the underlying liver disease or the presence of multiple HCCs contraindicate hepatic resection, liver transplantation (LT) may be considered under specific selection criteria [1,2]. The Milan criteria (one lesion < 5 cm; alternatively, up to three lesions, each <3 cm; no extrahepatic manifestations; no evidence of macrovascular invasion) currently represent the benchmark for the selection of patients with HCC for orthotopic LT [1,2]. The potential curative effect of LT for both the tumor and the underlying liver disease is responsible for the best therapeutic outcome for these patients, with a patient survival rate over 70% and tumor recurrence of 10-15% at 5-year follow up [1,2]. In those cases with a tumor limited to the liver and with no macrovascular invasion but unresectable and beyond the selection criteria for LT, trans-arterial chemoembolization (TACE) is considered the first-line treatment, either as a down staging strategy for LT indication or as a definitive therapy, with a reported median survival of 40 months [1,2].

The therapeutic management of HCC patients so far has been mainly based on surgery (hepatic resection and LT) or interventional radiology (ablation, TACE), rather than on systemic therapies which have usually been reserved for advanced-stage tumors [1,2]. Thus, the diagnostic work-up has been primarily targeted to assess tumor morphologic features such as nodules number, max diameter, presence of satellite nodules or macrovascular invasion, rather than precise biologic behaviour. As a matter of fact, liver biopsy is not routinely performed, and its indication is restricted to cases of nodules highly suspicious for malignancy but not completely meeting imaging criteria for HCC, or of non-cirrhotic patients with suspected HCC lesions [1,2]. The main reason for limiting liver biopsies in HCC is the risk of adverse events possibly impacting on the diagnostic and/or therapeutic pathway [8-10]. These variably comprise bleeding, bile leakage, and the potential risk of neoplastic seeding, although incidence is low [8-10]. In particular, the risk of tumor seeding along the needle path has been reported in less than 1% of cases, and its clinical impact in most cases was successfully controlled by resective or ablative treatments [8-10].

However, recent advances in our understanding of the biomolecular mechanisms implicated in the pathogenesis of HCC are now identifying potential therapeutic targets which may provide new and revolutionary therapeutic opportunities.

Sorafenib and Lenvantinib, multikinase inhibitors blocking 40 kinases including vascular endothelial growth factor receptor 2 (VEGFR2) and BRAF, are considered as targeted first-line therapies for patients with advanced HCC and those with intermediate-stage (BCLC B) disease not eligible for, or progressing despite locoregional therapies [1,2]. Other multikinase inhibitors that block the activity of protein kinases involved in angiogenesis, oncogenesis, and tumor microenvironment, such as Regorafenib and Cabozantinib, are considered as targeted second-line therapies for patients with advanced HCC who have tolerated Sorafenib but progressed. All these drugs have been shown to improve survival and decrease the relative risk of death compared with placebo in patients with advanced HCC not amenable by the standard-of-care curative therapies (resection, transplantation, or ablation) (Table 1) [1,2].

The recent success of checkpoint inhibitors in different tumors has stimulated several ongoing clinical trials of different checkpoint inhibitors in HCC [1,2]. Immunotherapy with Nivolumab—that targets PD-1—can be considered in patients who are intolerant to, or have progressed under approved tyrosine kinase inhibitors, demonstrating a response rate of 17% and time to progression of 6.5 months with median survival close to 16 months in second-line patients; this compares favourably with all the previously reported phase III second-line trials in HCC [1,2]. Meanwhile, a phase II trial of another anti-PD-1 antibody—pembrolizumab—as a second-line treatment (KEYNOTE-224) has recently been reported. The 16.3% response rate (RECIST v1.1) and 78% 6-month overall survival (OS) observed among the 104 patients studied are in line with the results seen with Nivolumab (Table 1) [1,2].

Adoptive T cell therapy (ATC) is an immunotherapeutic strategy targeted to specifically activate the host cellular immunity against tumor-associated antigens. It has

emerged as a highly effective treatment in the management of both hematological and virus-associated cancers [17]. On the other hand, solid tumors tend to show a high grade of resistance to ACT, mainly due to a genomic heterogeneity and instability resulting in loss of target-specific antigens expression, as well as due to a hypoxic, proinflammatory and immunosuppressive tumor microenvironment (TME) [17]. However, recent studies have highlighted that targeted therapies may not only inhibit molecular or biochemical pathways crucial for tumor growth and maintenance, but also disrupt the TME and enhance the local immune reactivity. Therefore, the combination of target therapies and ACT may represent the key point to significantly enhance the therapeutic outcome in the management of solid tumors [17].

A significant limitation in the HCC treatment is the absence of early diagnostic markers. Small tumoral masses with a diameter of less than 3 cm have over a 50% chance to be cured via surgical resection or thermal ablation. Nevertheless, the identification of these tumoral areas is still problematic due to the absence of pathognomonic symptoms in early stages of HCC. To overcome the problem, over the years, several laboratories have investigated molecules with a potential diagnostic impact as well as a possible therapeutic output when targeted. Among the most interesting categories of biomarkers studied have been oncogenes and tumor-suppressor genes [18]; plasma methylated DNA [19]; microRNA (miRNA) and long non-coding RNA (lncRNA) [20]. Oncogenes play important roles in promoting tumorigenesis and they are often upregulated epigenetically after liver damage. They can influence cell proliferation, invasion, migration, cell cycle dysregulation, and other tumor-related phenomena like metastasis formation. Being upregulated, they can be promising candidates in early HCC detection as well as good targets for silencing therapies. A recent example is the identification of APEX2 upregulation associated with a worse prognosis in overall liver cancer patients' survival [21]. Aberrant methylation of tumor-suppressor genes and demethylation of oncogenes are associated with HCC malignancy potential. Methylation regulation could thus be explored to target genes such as Sal-like proteins 4, often associated with chronic hepatitis B infection, but also for prognostic purposes, as seen by Wu et al. in 2017 [22].

Gene expression is regulated not only via methylation, but also via non-coding RNAs. A class of small non-coding RNAs, called miRNAs, is often dysregulated in cancers and could be used as excellent biomarkers thanks to miRNAs' expression stability and resistance to endogenous RNase. For example, miR-23a has been studied not only in relation to HCC screening tests but also in a prognostic scenario, dependent on the HCC stage, tumor size, and the presence of multiple focal lesions [23].

The aberrant biogenesis of lncRNAs has been implicated in the pathogenesis of HCC. LncRNAs are involved in many regulative processes through their binding with DNA, RNA, proteins, and small peptides, so once their expression profile is altered the impact on the cell physiology is evident. RNA-sequencing and microarray experiments have shown distinct lncRNA expression profiles in HCC tissue compared with non-cancerous ones [24]. Alteration in the epigenetic silence/activation or in the processing patterns of lncRNA with oncogenic function has been reported in HCC patients [25]. In this scenario, therapeutic strategies aimed to regulate the transcriptions levels of lncRNA may lead to positive results, as seen in RNA interference experiments [26].

Comprehensively, what emerges in literature is an increasing interest in the identification of biomarkers for both prognosis and therapy. Identification of early diagnostic markers able to prevent the development of HCC will lead to the consequent possibility to immediately target the tumor, limiting its development. A combinational approach could be useful to increase diagnosis accuracy, as in the case of the GALAD score, and to identify biomarkers that could lead to the development of new therapeutic strategies based on mechanisms of upregulation, downregulation, or delocalization of the biomarkers themselves [27-29]. Despite its promising possibilities, however, a therapeutic strategy based on the identification of effective biomarkers for HCC will be complex and will require a deep investigation of many mechanisms before

	Drug	Target	Clinical trial phase	Ref
First line treatments	Sorafenib	Multikinase inhibitor	FDA approved (2007)	[11]
	Lenvantinib	Multikinase inhibitor	FDA approved (2018)	[12]
Second line treatments	Regoraferib	Multikinase inhibitor	FDA approved (2017)	[13]
	Cabozantinib	Multikinase inhibitor	FDA approved (2019)	[14]
	Nivolumab	Antibody against PD-1	III*	[15]
	Pembrolizumab	Antibody against PD-1	II	[16]
*FDA approved Nivolumab in combination with Ipilimumab to treat advanced HCC in 2020.				

Table 1: Summary of small molecule inhibitors used in HCC clinical protocols.

treatment planning can start. Unfortunately, as we already discussed, another major problem in HCC treatment is the limited options available to treat patients in an advanced stage.

In this context, over the years our and other laboratories have been focused on the Apurinic/apyrimidinic endonuclease 1 (APE1) protein, which was proposed as a predictive marker in HCC. In a retrospective IHC study, the authors demonstrated that extra nuclear accumulation of APE1 correlated with a significant reduction of life expectancy in HCC patients [30]. More recently, Pascut et al. proposed the presence of APE1 in the serum of HCC patients as a new diagnostic biomarker and its role as a paracrine pro-inflammatory molecule which may modulate the inflammatory status of the cancer microenvironment [31]. APE1 is a nuclear/mitochondrial protein and it is a key component of the base excision repair (BER) pathway. The BER pathway is present both in the nucleus and the mitochondrial matrix and it is involved in repairing non-helix-distorting base lesions [32]. We demonstrated that APE1 is translocated to mitochondria upon oxidative stress, where it contributes in the stability of mitochondrial DNA (mtDNA) [33], and that its translocation relies on the mitochondrial import pathway MIA [34]. Recently, our laboratory more deeply investigated the trafficking and localization of APE1 during HCC staging. Our data revealed a mitochondrial localization of APE1 that was dependent on the Edmondson-Steiner grading and that inversely correlated with the levels of mtDNA damage. Grades 1 and 2 HCC patients showed a significantly higher expression of mitochondrial APE1, which accounted for lower levels of mtDNA damage observed in the tumor tissue with respect to the distal area. In contrast, the strong cytoplasmic positivity in Grade 3 was not associated with APE1's mitochondrial accumulation even when accounting for the higher number of mtDNA lesions measured [35]. Based on this evidence linking the mitochondrial localization of APE1 with lower levels of mtDNA damage during the early stages of HCC, a possible therapeutic approach based on the inhibition of mitochondrial APE1 trafficking and/or activity in combination with a DNA damaging agent is likely to be effective in slowing down tumor development. In this frame, our laboratory is developing a peptide-based approach to inhibit the pathway responsible for mitochondrial translocation of APE1.

In conclusion, although in recent years standardized diagnostic protocols and therapeutic procedures associated with new pharmacological approaches have enhanced the success rate extending the life expectancy of HCC patients, HCC remains one of the most prevalent neoplasia worldwide. Further studies are needed to discover reliable and easily accessible early diagnostic markers as well as new and more effective therapies.

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