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Decoding the molecular and genomic landscape of hepatocellular carcinoma: biomarker discovery, classification frameworks, and therapeutic targeting

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a major cause of cancer-related deaths worldwide. Conventional diagnostics lack sensitivity for early detection. This review summarizes emerging biomarkers, advanced molecular classifications, and technologies such as RNA sequencing and liquid biopsy that enhance tumor characterization and monitoring. While promising, these approaches face validation and accessibility barriers. Their integration into clinical practice could enable personalized therapy and improve outcomes.

Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related mortality worldwide, reflecting the limitations of current diagnostic and therapeutic strategies. Despite advancements in surveillance, traditional diagnostic tools such as ultrasound and serum markers like alpha-fetoprotein (AFP) lack sufficient sensitivity and specificity for early detection, resulting in late-stage diagnoses and poor clinical outcomes. Consequently, there is an urgent need to explore novel biomarkers and advanced molecular technologies to improve the early diagnosis and prognostic assessment of HCC.

In this review, we will discuss the latest advancements in biomarker discovery for HCC, focusing on innovative candidates that have demonstrated potential for clinical application. Particular attention will be given to novel molecular markers such as DCLK1, GPC3, CD276, and OPN, which have shown promising results in identifying early-stage HCC and predicting disease progression. Additionally, we will explore cutting-edge technologies like single-cell sequencing and liquid biopsy, which are revolutionizing our understanding of HCC biology and enabling more precise patient stratification.

Finally, we will address how these emerging biomarkers and technological innovations are contributing to novel molecular classification systems for HCC. By integrating these new discoveries with genetic and immunological profiling, it is now possible to redefine HCC subtypes with greater accuracy, paving the way for personalized therapeutic strategies. This review aims to provide a comprehensive overview of these advancements, highlighting their implications for clinical practice and future research directions.

HCC: epidemiology and diagnostic strategies applied in current clinical practice

Hepatocellular carcinoma (HCC) is the sixth most common neoplasia worldwide and the most common form of primary liver cancer, accounting for approximately 75–85% of cases. Originating from hepatocytes, the liver's main cells, HCC typically develops in the context of chronic liver disease, often associated with cirrhosis. Its incidence varies significantly across different geographic regions, reflecting the distribution of risk factors. Furthermore, its global incidence is on the rise, making it a major cause of cancer-related mortality worldwide[1–4].

Numerous risk factors have been identified for HCC. Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) is are main contributor, particularly in regions such as East Asia and sub-Saharan Africa

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for HBV, and Western Europe and North America for HCV[1,5]. Alcohol abuse, leading to fatty liver disease and cirrhosis, is another significant determinant. In recent decades, metabolic dysfunction-associated steatotic liver disease (MASLD), previously named non-alcoholic fatty liver disease (NAFLD), has emerged as the primary cause of chronic liver disease globally, reflecting global changes in lifestyle and dietary habits[1,6]. MASLD is also the primary driver of the rising incidence of HCC, with the annual occurrence of MASLD-related HCC projected to increase by 45–130% by 2030[7].

The highest risk of HCC is observed in patients with advanced fibrosis or cirrhosis. However, numerous studies have shown that 20–50% of HCC cases occur in individuals with MASLD, even in the absence of cirrhosis[8]. Finally, other risk factors for HCC include exposure to aflatoxins, carcinogenic substances produced by molds in poorly stored foods, and genetic conditions such as hereditary hemochromatosis[1,5]. The management of HCC is complex and requires a multidisciplinary approach. Major international guidelines, including those from the European Association for the Study of the Liver (EASL)[1] and the American Association for the Study of Liver Diseases (AASLD)[4], emphasize the importance of a regular biannual screening in high-risk patients, such as those with cirrhosis, using ultrasound and alpha-fetoprotein measurements. Abdominal Ultrasound (US) is the most commonly used surveillance tool for HCC due to its accessibility, low cost, and non-invasiveness. It is recommended as the first-line tool for its ability to detect liver nodules. However, the effectiveness of ultrasound is influenced by factors such as obesity and the presence of severe steatosis, which can limit adequate visualization of liver parenchyma. Additionally, the sensitivity of ultrasound depends on the operator's experience, which can lead to variability in results[1,4].

Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) are used to confirm the nature of suspicious lesions identified during ultrasound and to complete tumor staging. These exams are more expensive and require the use of contrast agents, which may not be suitable for patients with severe renal impairment[1,4]. Abbreviated MRI has been proposed as an alternative also for screening, but there are limited data to support its use, even when restricted to some specific subsets of patients[9]. Although less commonly used than MRI e CT, contrast-enhanced ultrasound (CEUS) represents a valuable diagnostic tool for HCC, particularly for patients for whom CT or MRI are not feasible. Its utility is largely attributed to the exclusive intravascular distribution of the contrast agent, which allows for real-time assessment of vascular dynamics and of HCC characteristic enhancement patterns[10].

Another HCC diagnostic tool is represented by tumor markers, such as alpha-fetoprotein (AFP). Although AFP is still widely used as a screening marker for HCC, its sensitivity and specificity are insufficient. Only about 50% of patients with HCC present elevated AFP levels, since some HCCs, particularly in the early stages, may not produce AFP at all[11,12]. On the other hand, AFP concentrations can be elevated in the absence of HCC, such as in patients with active hepatitis. Despite this, current guidelines suggest the combined use of US and AFP in HCC screening, since this approach translates into greater sensitivity with an acceptable reduction in specificity[1]. Notably, in the last EASL guideline, the use of lens culinaris agglutinin-reactive alpha-fetoprotein (AFP-L3), angiopoietin-2 (ANG2), and the combination of AFP with Des-gamma-carboxyprothrombin (DCP), has been proposed to improve the performance of AFP, but these new markers have significant limitations. AFP-L3 is a specific fraction of AFP more closely associated with HCC, but it is not sensitive enough to be used as a sole indicator. ANG2, a marker for angiogenesis, was proposed as a serum tumor biomarker despite it is less studied. DCP is a marker produced by tumor cells in HCC, but it can also be elevated in other non-tumor conditions, limiting its clinical utility[11,12]. Moreover, composite biomarker models, such as the GALAD score, have been developed. This score is the most thoroughly validated integrative tool, combining gender, age, AFP, AFP-L3, and DCP, demonstrating 82% sensitivity and 89% specificity, with an AUROC of 0.92 for HCC detection. Notably, even in early-stage HCC, it maintained acceptable performance (sensitivity 73%, specificity 87%)[13]. More recently, novel investigational models have emerged, such as the HCC Early Detection Screening (HES) score and the aMAP score. The original HES score combined AFP with age, alanine aminotransferase, and platelet count[14]. The updated HES v2.0, which incorporates AFP-L3 and DCP, has shown a 6–15% higher sensitivity than GALAD during 1–2 years of surveillance[15]. Finally, aMAP score, which integrates age, sex, albumin-bilirubin, and platelet count, is currently under investigation as a risk stratification tool for personalized HCC surveillance in patients with chronic liver disease[16].

Histological features of HCC and molecular correlates

Morphologically, HCC is characterized by hepatocytic differentiation, which can be identified through routine microscopy and supported by immunohistochemical and molecular analyses. HCC disrupts the normal hepatic architecture, primarily through the absence of portal tracts and the distortion or loss of the reticulin framework[17]. These architectural changes result from tumor expansion and the replacement of normal hepatic parenchyma with malignant cells. HCC demonstrates four principal histological growth patterns. First, the trabecular pattern, the most common growth pattern, is characterized by malignant hepatocytes arranged in broad plates or cords, usually more than three cells thick. These trabeculae are separated by sinusoids, which may show endothelial wrapping or transgressing vessels[18]. Second, the solid pattern, composed of densely packed tumor cells with minimal intervening stroma or sinusoidal spaces. This pattern is frequently associated with high-grade tumors and poor differentiation[18]. Third, the pseudo-glandular (pseudo-acinar) pattern, in which tumor cells form gland-like structures resembling acini, which may contain bile or necrotic debris. This pattern highlights the ability of malignant hepatocytes to mimic glandular differentiation[18]. Fourth, the macrotrabecular pattern, characterized by thick trabeculae (≥ 10 cells), is often associated with an aggressive clinical course and worse prognosis[18]. Mixed patterns are observed in about 50% of cases, emphasizing the histological heterogeneity of HCC. This heterogeneity can reflect tumor progression or dedifferentiation.

The tumor often shows increased arterialization, characterized by the presence of abnormal arterioles within the parenchyma and sinusoidal capillarization. This vascular transformation is a hallmark of HCC and reflects the dependency of the tumor on arterial blood supply. Sinusoids within the tumor show capillarization, with endothelial cells expressing markers like CD34, reflecting a shift from normal hepatic to tumor-associated vasculature. Small aggregates of macrophages or peliosis-like areas may also be present within the tumor sinusoids, further highlighting its complex microenvironment[19].

The World Health Organization (WHO) 2019 classification recognizes several histological subtypes of HCC, each with distinct clinical and molecular features[20]. In their review, Choi and Thung described very well all HCC subtypes divided for histological and mutational features[20]. These subtypes include fibrolamellar carcinoma, macrotrabecular massive HCC, scirrhous HCC, clear cell HCC, and chromophobe HCC, among others; the full list is available in Table 1, and in Fig. 1 shows histological features of some HCC subtypes. A close connection exists between HCC subtypes and specific genetic mutations, which influence their clinical behavior and prognosis. Fibrolamellar carcinoma, for instance, occurs predominantly in younger patients without underlying liver disease and is characterized by eosinophilic tumor cells and dense intratumoral fibrosis with a lamellar pattern. Molecularly, it involves the DNAJB1::PRKACA fusion gene. Scirrhous HCC, characterized by abundant fibrosis, is often associated with mutations in the TSC1/2 genes and activation epithelial-to-mesenchymal transition signaling pathway. Similarly, chromophobe HCC is linked to the alternative lengthening of telomeres (ALT) mechanism, which allows telomere maintenance independent of telomerase reverse transcriptase (TERT) promoter mutations. Macrotrabecular massive HCC, defined by a growth pattern of thick trabeculae (≥10 cells), is linked to mutations in tumor protein p53 (TP53) and fibroblast growth factor (FGF) 19 amplifications correlating with its aggressive clinical course and high vascular invasion rates, often signifying a poor prognosis. In contrast, clear cell HCC, enriched in

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Table 1 | Macro-, micro- histologica and genetic features of different HCC subtypes

Subtype of HCC	Macroscopical features	Prelevance	Histology features	Markers	Mutation
Steatohepatitic HCC	Steatohepatitic HCC subtype shows a multinodular confluent, yellow–gray mass imparted by the presence of steatosis and fibrosis, respectively, within the tumor.	5-20%	This subtype displays a similitude at least 50% with to steatohepatitis, showing macrovesicular steatosis, lymphocytic inflammation, balloon cells, Mallory–Denk bodies, and pericellular fibrosis.		This subtype presents mutation at level of catenin beta 1 at late stage[95], tumor protein p53 (TP53)[96], and telomerase reverse transcriptase (TERT) promoter[97].
Clear Cell HCC	Clear Cell HCC subtype presents a grayish nodular mass, surrounded by a fibrous capsule, into the cirrhotic liver.	3-7%	This subtype is characterized by middle or high differentiated tumor cells showing clear cytoplasm in over 80%, and steatosis features are observed in one-third of HCC. It could be possible to detect tumor cells containing lipid drops.	This subtype shows positivity for hepatocyte paraffin 1 (Hep Par-1), arginase-1[98].	This subtype presents mutation at level of isocitrate dehydrogenase (NADP(+)) 1 (IDH1)[99].
Macrotrabecular massive HCC	Macrotrabecular massive HCC subtype is charaterized by multiple brown-tan nodules bulge over the surrounding flat fibrous strands.	5%	This subtype exhibits a large quantity over 50% of macrotrabecular growth tumor pattern (the most accepted cutoff: >6 and ≥10 cells thick) with tumor cells associated with high-grade nuclear atypia.	This subtype shows positivity for alpha Fetoprotein (AFP) serum[100], Cluster Differentiation (CD) 34[101], endothelial cell-specific molecule 1 (ESM1)[102], vessels that encapsulate tumor clusters (VETC)[100].	This subtype shows mutation at the level of TP53, and overproduction of fibroblast growth factor (FGF) 19, ataxia-telangiectasia mutated protein (ATM), angiopoietin 2 and vascular endothelial growth factor A (VEGFA)[100].
Scirrhous HCC	Scirrhous HCC subtype displays abundant intratumoural fibrous stroma (at least 50% of tumor).	4%	This subtype shows widespread intratumoural fibrosis over 50% of HCC and well and/or moderate differentiated tumor cells. It could be possible to appreciate hyaline bodies during histological analysis.	This subtype shows positivity for arginase-1 and glypican-3 in ~80% of cases. At the same time, around the 50% of cases are Hep Par-1 negative. Epithelial cell adhesion molecule (EpCAM), CK7, and CK19 are commonly detected[103,104].	This subtype displays mutation in TSC1/TSC2 genes and enhances activation epithelial-to-mesenchymal transition signaling pathway[105].
Chromophobe HCC	Chromophobe HCC subtype presents a well-circumscribed and encapsulated nodule with having white-yellowish appearance	3%	This subtype manifests clear to pale eosinophilic cytoplasm and mostly uniform color nuclei; cystic spaces loaded with a serum-like solution are often present. Moreover, it could be possible to observe abrupt focal areas presenting tumor cells with greater nuclear anaplasia.	This subtype shows positivity for Alternative lengthening of telomeres (ALT)[106]	
Fibrolamellar HCC	Fibrolamellar HCC subtype displays multilobulated and well-circumscribed nodules; moreover, a central stellate scarring area is present.	1%	This subtype displays neoplastic hepatocyte cells with intratumoral dense fibrosis, deposed in parallel or lamellar bands. Commonly, it could be possible to detect pale bodies, hyaline bodies, and pseudo-glands. Sometimes calcification, intratumoral cholestasis, mucin production, and copper accumulation were revealed.	This subtype shows low positivity for AFP serum (<200 ng/mL), Hep Par-1, co-expression of cytokeratin (CK) 7 and CD133 and CD44[107].	This subtype exhibits microdeletion in the chromosome 19 comporting DnaJ heat shock protein family (Hsp40) member B1 (DNAJB1):: protein kinase cAMP-activated catalytic subunit alpha (PRKACA) fusion gene[106,108].
Neutrophil-rich HCC	Neutrophil-rich HCC subtype presents a capsule showing a white and heterogeneous cleavage surface.	<1%	This subtype is characterized by the high number of tumor-infiltrating neutrophils and HCC tumor cells poorly differentiated.	This subtype shows strong positivity for granulocyte colony-stimulating factor (GCSF), and it shows high serum levels for interleukin (IL) 6 and C-reactive protein[106,109].	
Lymphocyte-rich HCC	Lymphocyte-rich HCC is well-circumscribed with a color reddish brown to tan.	<1%	This subtype displays a higher number of intratumoral lymphocyte cells than HCC tumor cells. It could be possible to appreciate pale bodies into HCC tumor cells.	This subtype shows positivity for Hep Par-1 and arginase-1[110].	

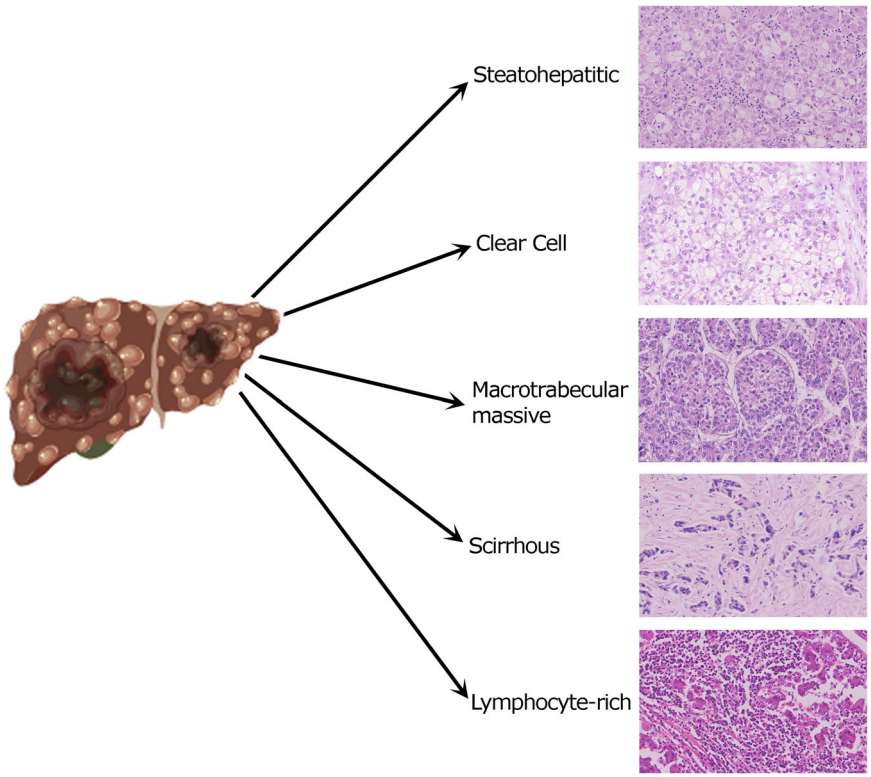

Fig. 1 | HCC histological subtypes. The most common HCC subtypes are represented (courtesy of Prof. Simone Carotti and Dr. Andrea Baiocchini). All images are taken with NanoZoomer digital scanner, magnification 40X, linear bar 50 µm. Created with BioRender.com (https://BioRender.com/3fnrku2).

Table 2 | A summary of detection ways for the proposed new biomarkers

Marker	Proposed detection				
	IHC / IF staining	Plasma	PET/ immunoPET	gene expression	Immuno cells
DCLK1	X[19–22]	X[17,31]		X[20,27]	X[18,29,33–35]
GCP3			X[33]		X[38]
CD276	X[40]				
FGF19	X[45]	X[46]			
INMT	X[51–53]			X[51–53]	
MMP10	X[56,57]			X[56,57]	
DIKK1		X[58,59]			
OPN	X[66,67]	X[57,58]			

glycogen, exhibits fewer genetic alterations, contributing to its relatively favorable prognosis compared to other subtypes. Notably, immunohistochemistry (IHC) plays a pivotal role in diagnosing HCC and its subtypes. Markers such as arginase-1, Hep Par-1, and glypican-3 assist in identifying hepatocytic differentiation. Subtypes like scirrhous HCC, characterized by dense fibrosis, may express cytokeratin (CK) 7 and CK19, markers usually associated with cholangiocarcinomas.

The histological and molecular diversity of HCC underscores the importance of comprehensive diagnostic approaches. Mixed histological patterns, seen in about half of HCC cases, reflect tumor progression and heterogeneity. Molecular alterations often accumulate during this progression, highlighting the dynamic nature of HCC. Understanding these changes is vital for advancing treatment strategies, including precision medicine.

However, we currently lack effective tools for the diagnosis and prognosis of early-stage HCC. For this reason, it is necessary to explore new biomarkers or to gain a better understanding of, and perhaps use differently, the current clinical biomarkers described in the guidelines.

New putative clinical biomarkers for an early diagnosis of HCC

As mentioned above, current biomarkers and assays used in clinical practice are insufficient both to discriminate patients with HCC from those with pre-tumoral liver disease and to detect early HCC recurrence after surgical or medical treatment. For this reason, in recent years, several new biomarkers have been proposed with the aim to improve HCC prediction, screening, or monitoring. In this chapter, we will focus on new putative HCC biomarkers derived in recent years from studies on animal models and/or patient cohorts. (Table 2 and Fig. 2).

Doublecortin-like kinase 1

Doublecortin-like kinase 1 (DCLK1) is a protein associated with microtubules in cytoplasm, involved principally in the regulation of microtubule polymerization and neurogenesis[21,22]. Several research teams described the overexpression of DCLK1 in different solid tumor type[23–29]. A strong relationship has been demonstrated between overexpression of DCLK1 and several tumor processes including tumor growth, metastasis, epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSCs) self-renewal, tumoral microenvironment regulation[21,22,30–32]. Moreover, high DCLK1 is observed in patients with gastrointestinal tumors that have a worse prognosis[33–36]. Previous studies have linked increased DCLK1 level expression with poor prognosis in HCC, suggesting its potential as a biomarker for diagnosis and monitoring of disease progression. It was demonstrated that DCLK1 is targeted by microRNAs (miRNA) like miR-144 and miR-200a, which suppress DCLK1 expression, inhibiting cancer cell growth, stemness, and invasion. On the other hand, DCLK1 knockdown upregulates tumor suppressor miRNAs such as miR-143 and miR-145, reducing cell proliferation and migration[37]. Moore et al. have identified that miR-1246 and miR-184 were upregulated, while miR-206 was downregulated, in patients with high DCLK1 sera levels. Authors suggested that elevated DCLK1 and miR-1246 levels are associated with inflammation-driven tumorigenesis, while miR-206 reduction facilitates the transition from cirrhosis to HCC, and this could be used in clinical practice for patients suffering of chronic liver disease as biomarkers for following the progression

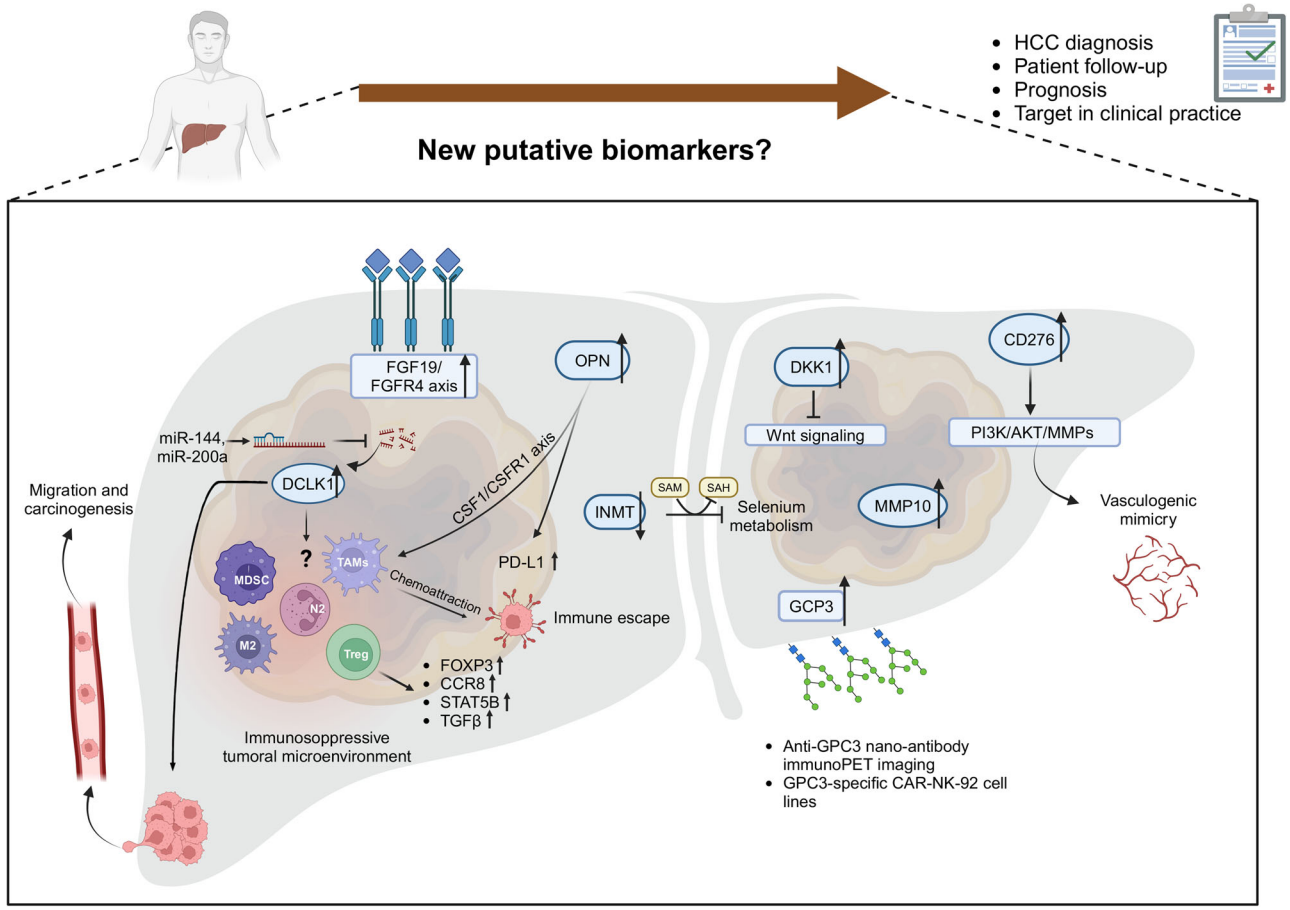

Fig. 2 | Conceptual map of candidate biomarkers in hepatocellular carcinoma. Schematic representation of proposed biomarkers for HCC diagnosis and prognosis, as summarized in the present review. Created with BioRender.com (https://BioRender.com/5xqg3hc).

from pre-neoplastic conditions to HCC and/or offer therapeutical targets[37]. Recent research associates DCLK1 with immune cell infiltration in cancers like gastric and colon cancer, particularly with tumor-associated macrophages (TAMs) and regulatory T cells (Tregs)[33,38,39]. Velazquez-Enriquez et al. have demonstrated that high DCLK1 expression in HCC patients correlates with increased infiltration of immune cells such as B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, dendritic cells, and myeloid-derived suppressor cells (MDSCs). Furthermore, DCLK1 shows significant correlations with Treg markers, including FOXP3, CCR8, STAT5B, and TGFB1, suggesting a role in promoting an immunosuppressive tumor microenvironment. Authors concluded that this interaction could help tumors evade immune surveillance, highlighting DCLK1 as a crucial regulator in immune suppression and a potential target for therapeutic intervention[22]. The possibility of monitoring HCC onset and progression through a simple serum analysis is very appealing. However, the mechanisms and functional role of DCLK1 need to be investigated more thoroughly before assuming a clinical role in liver cancer.

Glypican 3

Glypican 3 (GPC3) is a heparan sulfate proteoglycan that is rarely expressed in normal tissues; however, it is overexpressed in HCC and plays a role in HCC development[40–42]. In fact, GPC3 is overexpressed during the aggressive progression of HCC, indicating that it may play a role in the development of HCC[41]. Based on this, An et al. [40] have used anti-GPC3 nano-antibodies in immune-positron emission tomography (immunoPET) imaging to increase the diagnostic sensitivity for HCC, obtaining promising results in HCC animal models. However, tracers derived from nano-antibodies have the problem of being widely absorbed by the kidneys and this could compromise their clinical translation[40]. Another method to use the different expression of GPC3 was described by Cao et al. They developed two glypican-3 (GPC3)-specific CAR-NK-92 cell lines (GPC3-CAR-NK), GC33-G2D-NK and GC33-CD28-NK, and analyzed their effect in vitro and in vivo when used in combination with microwave ablation (MWA)[42]. Between the two kinds of engineered cells, GC33-G2D-NK showed a better degranulation upon stimulation and synergic antitumor effect with MWA against HCC[42]. The strength of this approach lies in the fact that MWA is already used in clinical practice, and clinical trials have been conducted using GPC3-specific CAR-NK cells[43]. The limitation of the study by Cao et al. was the use of NSG mice, since they lack a functional immune system, therefore preventing the interaction between CAR-NK cells and innate immune cells[42]. Li et al.[41] performed a retrospective analysis based on GPC3 expression, demonstrating that MRI, radiomics, tumor morphology, and microvascular invasion (MVI) can noninvasively predict GPC3 expression in HCC patients[41]. Authors suggested integrating these indicators with clinical factors into nomograms to offer valuable insights for tailoring personalized treatment plans for patients diagnosed with HCC prior to surgery[41].

The ability to both detect the presence of HCC and use specific NK cells to eliminate cancer cells makes GPC3 a promising clinical marker. However, further studies are needed before GPC3-oriented CAR-NK cells can be used in clinical practice.

CD 276

Cluster of differentiation (CD) 276, or B7-H3, is the principal member of B7 superfamily. PD-L1 (B7-H1) is another member of this superfamily[5,44]. In normal human tissues, CD276 is broadly expressed at the mRNA level but its protein expression is typically limited, suggesting post-transcriptional regulation. At the same time, abnormal CD276 expression is seen in several

human cancers, such as HCC[45]. Growing evidence suggests that CD276 plays a significant role in both innate and adaptive immunity, as well as in tumor aggressiveness. It is expressed in immune cells like macrophages and APCs, regulating T cell function and contributing to cancer cell migration and invasion[46]. In fact, data obtained in vitro and in vivo by Liu et al. have demonstrated that, in HCC patients, high levels of CD276 are associated with poor prognosis, modifications in immune cell infiltration, immune marker expression, and macrophage polarization[5]. Authors concluded that CD276 could be a possible prognostic marker and play a role as a putative target for immunotherapy in HCC. Furthermore, Cheng et al. have described a new pivotal role of CD276 in HCC. Based on their data, CD276 promotes vasculogenic mimicry formation in HCC via the PI3K/AKT/MMPs pathway[44]. Vasculogenic mimicry is a process put in action by tumor to obtain sufficient blood supply without involving endothelial cell. This process is one of the hallmarks of cancer and it is closely linked to invasion, metastasis and poor outcome[47]. Interestingly, CD276 can activate matrix metalloproteinases (MMPs), in particular MMP14/MMP2 and MMP2/MMP9, suggesting that it can both regulate tumor aggressiveness and the EMT process and improve vasculogenic mimicry formation[44].

Unfortunately, CD276 can be detected only by mRNA assay, and this nullifies its use as a serum marker. However, its potential as a therapeutic target remains of interest, since interfering with the EMT process and tumor invasion could have significant antitumor effects.

FGF19

Fibroblast growth factor (FGF) 19 is a member of FGFs family. When these proteins link their receptor, the FGF receptor (FGFR) tyrosine kinase can regulate multiple biological processes such as cell growth, differentiation, angiogenesis, and metabolism[48–51]. The principal receptor for FGF19 is FGFR4, that is able to form a selective linking[52]. In healthy conditions, FGF19 regulates bile acid synthesis and nutrient metabolism[48]. Several studies have demonstrated that the FGF19-FGFR4 axis plays a role to promote tumorigenesis, and high expression of FGF19 indicates a poor prognosis in several cancers, including primary liver cancers[48–51]. In 2012, Miura et al. suggested HCC expression of FGF19 mRNA as an independent prognostic factor for overall survival and disease-free survival[53]. Using the Cancer Genome Atlas (TCGA) database, around 7% of HCC patients were found to have a high copy number of FGF19, and these patients showed shorter median survival compared with the low-expression cohort[54]. To evaluate Fisogatinib (BLU-554) in a phase I study, Kim et al. have divided their patients on the basis of FGF19 expression measured by IHC assay. Interestingly, Authors demonstrated that FGF19 is expressed in HCC tissues, but it was not detected in adjacent normal liver[49]. Consistently, Maeda et al. reported that serum FGF19 levels are significantly elevated in HCC patients compared to healthy subjects and patients with chronic liver disease. However, serum FGF19 levels alone showed diagnostic performance comparable to that of AFP or DCP levels. Remarkably, when the authors analyzed these three serum biomarkers together, they achieved high sensitivity, suggesting that this combination could aid in the diagnosis of HCC, particularly in cases of small tumors[50].

These findings highlight the potential of FGF19 as a biomarker for HCC diagnosis, especially when used in combination with other biomarkers like AFP and DCP. However, additional studies should be performed to validate its clinical use.

INMT

Indolethylamine N-methyltransferase (INMT), also known as amine N-methyltransferase, is an enzyme that catalyzes the methylation of thioether and selenoether compounds using S-adenosylmethionine (SAM) as a methyl donor[55,56]. Recently, it has been proposed as a prognostic marker due to the progressive decrease in its mRNA and protein levels across liver cancer stages. In fact, two independent works studying two different pathways have detected a strong INMT mRNA reduction in both murine and human HCC samples, confirmed by using data deposited in The Cancer Genome Atlas (TCGA) database[55,56]. Torres et al. studied DNA damage induced by diethylnitrosamine in the liver, promoting carcinogenic effects mediated by Nuclear Factor Erythroid 2–Like 2 (NRF2). During their analysis, the authors found that INMT was significantly downregulated in both animal models and human tissues. Moreover, INMT mRNA levels, obtained from the TCGA database, were closely associated with poor overall survival in patients[55]. Similarly, Sun et al. mRNA analysis indicated that low INMT levels correlate with poor prognosis and overall survival of HCC patients[56]. In their studies, Authors also confirmed that INMT expression is low in liver cancer, and, in in vitro experiments, they demonstrated that colony formation and cell proliferation were strongly increased by knock-down of INMT[56]. Sun et al. have also studied the role of INMT in hepatic selenium metabolism. Selenium is a fundamental mineral for human health, and the liver is the principal organ that suffers from selenium deficiency, being selenium levels negatively associated with liver diseases[57].

Interestingly, the same enzyme, analyzed in two different metabolic contexts, shows consistent results in HCC patients, suggesting that INMT could serve as a prognostic marker for HCC. However, the underlying molecular pathways remain unknown.

MMP10

Matrix metallopreonases (MMPs) are proteases capable of degrading all kinds of extracellular matrix proteins. Their role as tumoral microenvironment modulators is widely documented[58]. MMP10, strongly upregulated in liver injury conditions, is able to degrade several components of the extracellular matrix and it is capable to activate other MMPs[59]. Recently, this molecule has been correlated with HCC prognosis and overall survival by gene analysis of several human cohorts[60,61]. García-Irigoyen et al. firstly demonstrated that MMP10 plays a role in HCC development, tumor angiogenesis and tumor growth. Furthermore, Authors demonstrated that MMP10 is involved in the C-X-C chemokine receptor-4 (CXCR4) / stromal-derived factor-1 (SDF1) axis, enhancing metastasis and progression processes by the mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) 1/2 (MEK-ERK1/2) pathway[59]. In this same study, increased HCC MMP10 mRNA and protein expression were correlated with poor patient prognosis[59]. A similar result was obtained by Liu et al.[61]. Using TCGA and cBioportal as Cancer Genomics databases, they identified six genes, including MMP10, as useful to predict overall survival of HCC patients[61]. A different approach was used by Shaglouf et al., who focused on the differential expression protein and mRNA expression in liver of Wistar rats in which they induce HCC by administration of diethyl nitrosamine (DEN) and 2-acetylaminofluorene (2-AFF) compared to control rat, and the confirmed their results by analyzing human HCC samples[60]. By these means, Authors found that MMP10, among other molecules, plays a pivotal regulatory role in HCC development and progression[60].

Although the role of MMP10 in HCC has only been described in recent years, the literature consistently supports its major involvement in HCC progression and its potential use as a prognostic marker and predictor of overall survival. However, its assessment -whether through mRNA expression or immunohistochemistry (IHC)- requires a biopsy sample for analysis. Furthermore, as suggested by Liu et al., these findings should be validated in independent cohorts.

DKK1

Dickkopf-1 (DKK1) is a small protein, widely recognized for its role as an inhibitor of the Wnt/β-catenin pathway. Depending on the cell lines used in in vitro experiments, conflicting views exist regarding its oncogenic or tumor-suppressive function[62–64]. Interestingly, in 2011, Tung et al. proposed DKK1 as a potential serum diagnostic and prognostic biomarker for HCC patients. Moreover, DKK1 levels have been observed to increase with the severity of liver disease and the progression of HCC tumor stage[55], although it did not emerge as an independent prognostic marker. However, DKK1

played a pivotal role in tumor formation and growth in a mouse animal model[55]. A major limitation of this study was the small sample size analyzed. The following year, in 2012, Shen et al. expanded the sample size and used AFP as a control and validation model[56]. Their data suggest that DKK1 could be a useful biomarker for early HCC diagnosis; however, this serum marker is not capable of distinguishing between early-stage HCC and advanced HCC. Nevertheless, the authors proposed that DKK1 could aid in the early diagnosis of patients who are AFP-negative[56]. Interestingly, Chen et al. investigated the role of DKK1 in HCC invasion and migration. Their findings revealed that DKK1 plays a fundamental role in promoting invasion and migration processes in HCC through the β-catenin pathway, particularly by modulating MMP7, a downstream target gene[57]. Supporting this evidence, Suda et al. recently demonstrated that DKK1 contributes to remodeling the HCC tumor microenvironment by influencing angiogenesis[58].

The main limitation regarding the clinical use of DKK1 in the diagnosis and/or prognosis of HCC stems from its controversial biological role, which could be at least partially attributed to the different types of cell lines used in experiments in available studies. Nonetheless, in recent years, many researchers have focused on identifying the pathways and specific molecules involved, aiming to better understand the potential clinical applications of DKK1.

OPN

Osteopontin (OPN) is a glycoprotein involved in various physiological and pathological processes, including inflammation, immunity, and tumor progression[65]. Using a small Egyptian cohort, Abdel-Hafiz et al. demonstrated that OPN is overexpressed in HCC serum samples compared to healthy subjects. Moreover, authors observed a correlation between OPN levels analyzed by IHC and tumor grade[66]. Interestingly, OPN levels were negatively correlated with liver function, making the authors suggest OPN as a prognostic biomarker[66]. In the same period, Zhu et al. used a bigger Asian cohort and showed that OPN could be a good biomarker for HCC monitoring. In fact, authors detected serum OPN in HCC patients with tumor smaller than 2 cm, showing a higher sensitivity with respect to AFP[67]. The limitation of both cohorts was that most patients had HBV-related liver disease.

Several research teams have demonstrated the prognostic value of OPN in HCC. High OPN expression has been linked to poor prognosis, as it is involved in promoting angiogenesis, tumor cell migration, and invasion—key processes in cancer metastasis[68,69]. Wang et al. analyzed HCC tissues by HCC obtained from the surgery resection of patients without chemo- and/or radio-therapy treatment. By their data, OPN levels in HCC patients correlated with increase vascular invasion, tumor stages, reduced overall survival and disease-free survival[70]. As a confirmation of these data, Zhu et al. proved that mice KO for OPN are more resistant to HCC development than WT mice when exposed to DEN and carbon tetrachloride[71]. In their work, authors demonstrated the connection between macrophage migration, programmed death ligand 1 (PD-L1) expression, and OPN in a mouse animal model. This occurs through activation of the colony-stimulating factor-1 (CSF1) – CSF1 receptor (CSF1R) pathway in macrophages[71].

All available human data support the potential use of OPN as a clinical serum biomarker for the early diagnosis and prognosis of HCC. However, the limited cohort sizes and the predominance of HBV-associated HCC cases highlight the need for further studies before recommending OPN for clinical application. Concurrently, clinical observations and correlations with OPN levels in HCC tissues underscore its crucial role in HCC malignancy, tumor formation, and metastasis.

RNA sequencing

Over the past decade, advancements in single-cell sequencing technologies have revolutionized our understanding of cancer biology, particularly in the context of HCC. HCC is characterized by significant intratumoural heterogeneity, which complicates diagnosis, prognosis, and treatment. Recent studies utilizing single-cell RNA sequencing (scRNA-seq) have revealed distinct subpopulations of cells within HCC tumors, including various types of cancer cells, immune cells, and stromal cells. Based on this idea, Zhang et al. performed a landmark study, using single-cell sequencing to classify HCC tumors into distinct subtypes based on their immune microenvironment[72]. Moreover, Zheng et al. identified a unique subset of HCC cancer stem cells (CSCs) that exhibit high levels of stemness and are associated with poor prognosis. These CSCs are thought to drive tumor initiation, progression, and resistance to therapy, making them crucial targets for prognostic assessment and therapeutic intervention[73]. Ma et al. demonstrated that scRNA-seq could help identifying distinct gene expression signatures in HCC that are not detectable by conventional methods. These signatures include markers associated with tumor aggressiveness, metastatic potential, and immune evasion. Authors found that vascular endothelial growth factor (VEGF) could play a key role in driving HCC progression[74]. Several researchers have focused their work on the immune landscape of HCC as a prognostic model. Indeed, the analysis of immune populations in HCC reveals significant heterogeneity of immune cells within the tumor microenvironment, encompassing diverse subsets of T cells, macrophages, and natural killer cells, each contributing uniquely to tumor progression or suppression[73,74].

However, this technology is not without significant limitations. The enzymatic or mechanical tissue dissociation required for scRNA-seq can introduce transcriptional artifacts and lead to a biased representation of the tissue, often causing the underrepresentation of fragile cell types such as mature hepatocytes. A major drawback is the complete loss of spatial information, which prevents the analysis of how different cell types are organized and interact within the tumor microenvironment (TME)[75,76]. Furthermore, standard library preparation protocols based on poly-A selection exclude non-polyadenylated transcripts (e.g., certain non-coding RNAs) that may have crucial regulatory roles in cancer progression. To overcome some of these challenges, newer methodologies have emerged. Single-nucleus RNA sequencing (snRNA-seq) bypasses the harsh dissociation step by profiling RNA from nuclei isolated from fresh or frozen tissue. This preserves a more accurate representation of cellular diversity and allows for the capture of cell types and states that are often lost during scRNA-seq protocols[77]. In parallel, spatial transcriptomics technologies are directly addressing the need for spatial context. While often providing less transcriptional depth compared to single-cell methods, they map gene expression directly onto the tissue architecture. This allows for the study of cellular "neighborhoods" and the identification of spatially defined functional niches within the TME. Advanced imaging-based spatial methods, such as Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH), provide highly multiplexed, spatially resolved single-cell analysis at subcellular resolution[78]. The true frontier now lies in the multimodal integration of these technologies. By computationally combining the deep molecular profiling of scRNA-seq or snRNA-seq with the spatial maps from transcriptomics, it is possible to achieve a unified view that links cellular identity and state with a precise location within the tissue. Computational tools like Tangram or Cell2location enable this integration, allowing for the resolution of complex cell-cell interactions, the understanding of the TME's functional logic, and the discovery of regional biomarkers[76,79]. This integrated approach is essential for advancing diagnostic and therapeutic strategies in HCC.

Liquid biopsy

Liquid biopsy refers to the analysis of tumor-derived components, such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), microRNAs (miRNAs), and exosomes, present in the blood or other body fluids[80]. ctDNA is fragmented DNA released into the bloodstream by apoptotic or necrotic tumor cells. It carries tumor-specific genetic and epigenetic alterations, making it a valuable biomarker for cancer diagnosis and/or prognosis[80]. Studies have demonstrated that ctDNA can detect genetic mutations commonly associated with HCC, such as those in the TERT promoter, TP53, and CTNNB1 genes[80–82]. Additionally, hypermethylation of the RASSF1A and SEPT9 genes in ctDNA has shown promise in

distinguishing HCC patients from those with benign liver diseases or healthy controls[83,84]. CTCs are rare cancer cells that have shed from the primary tumor into the bloodstream[80]. In HCC, the presence of CTCs has been associated with advanced disease stages and poor prognosis. The molecular profiling of CTCs can reveal key mutations, especially those expressing epithelial-mesenchymal transition (EMT) markers, and phenotypic characteristics that may aid in the early detection of HCC[80,85–87]. miRNAs are small non-coding RNAs that regulate gene expression and play critical roles in cancer development and progression[80]. Pellizzaro et al. have identified miR-122 highly expressed in HCC patients when compared to healthy individuals, suggesting it as a prognostic marker[88]. Moreover, Jin et al. found that four miRNAs (miR-1972, miR-193a-5p, miR-214-3p and miR-365a-3p) are upregulated in HCC patients and that their elevated expression could be used to distinguish them from controls[89]. Exosomes are small extracellular vesicles that carry proteins, lipids, and nucleic acids, including miRNAs, and play a role in cell-cell communication[80]. Xue et al. have demonstrated that elevated levels of exosomal miR-93 are linked to poor prognosis in HCC patients[90].

Liquid biopsy represents a transformative approach in the diagnosis and prognosis of HCC, offering a non-invasive, real-time method to capture tumor dynamics. Beyond diagnosis and prognosis, liquid biopsy may also play a crucial role in guiding therapeutic decisions. ctDNA and CTC analysis can help stratify patients based on actionable genetic alterations, which may influence sensitivity or resistance to systemic therapies, including tyrosine kinase inhibitors and immune checkpoint inhibitors.

However, the sensitivity and specificity of liquid biopsy assays still require improvement to ensure reliability in clinical practice, along with further standardization and validation in large cohorts. A major challenge remains the difficulty of linking detected biomarkers to specific pathophysiological processes occurring within the liver and assigning them to distinct cellular subtypes, which limits their mechanistic interpretation[80].

In this context, extracellular vehicles (EVs), including exosomes, represent a promising tool as they carry cell-specific molecular cargo (e.g., proteins, lipids, and nucleic acids) that may help trace the cellular origin of the signal, potentially bridging the gap between circulating biomarkers and intrahepatic oncogenic events[80].

To fully leverage these biomarkers, it is crucial to integrate them within a broader framework of molecular and genetic classifications. By combining these novel markers with insights into HCC's molecular subtypes, we are going to enhance our understanding of tumor heterogeneity, optimize patient stratification, and develop more targeted therapeutic approaches.

Molecular and genetic classification of HCC

Although promising roles as molecular biomarkers are described in this review, no one is still applied in clinical practices for the management of HCC. Traditional classification based on histology and staging has proven insufficient in capturing the molecular complexity of this neoplasia, which significantly influences patient prognosis and treatment outcomes[91]. Based on this, several teams focused on classifying HCC based on its gene and molecular expression in order to suggest different treatments for different clusters of HCC patients. Gene expression profiling has emerged as a key tool in distinguishing different molecular subtypes of HCC. Techniques such as RNA sequencing and high-throughput microarrays enable comprehensive insights into gene activation and suppression patterns across HCC tumors, distinguishing molecular characteristics associated with various oncogenic processes[92]. Numerous studies have proposed classification systems based on the expression of genes related to cell cycle regulation, immune response, metabolic pathways, and oncogenic signaling[91–93]. Molecular classification based on gene expression has identified several subtypes, each linked to distinct biological processes and clinical trajectories, including cell cycle dysregulation, immune response variations, and metabolic changes. Recent advances in genomic technologies, particularly high-throughput gene expression profiling, have revolutionized HCC classification, leading to the identification of distinct molecular subtypes that offer better insights into disease pathogenesis and therapeutic vulnerabilities[91–93]. While molecular classification allows for nuanced understanding and targeted intervention, implementing these insights into clinical practice remains challenging. High costs, the need for complex bioinformatics support, and the rapid evolution of molecular profiling technology present obstacles in making these classifications universally available. Nonetheless, these classifications have shown potential in guiding more personalized treatment, aiding in early-stage patient stratification, and enabling closer monitoring for relapse risks[93]. For instance, the inflammation subtype and the immune class subtype of HCC, characterized by high expression of immune checkpoint molecules, are more likely to respond to immune checkpoint inhibitors. Conversely, tumors with Wnt/β-catenin activation exhibit an immunosuppressive microenvironment and may be resistant to ICIs. Therefore, molecular classification can serve as a valuable tool for patient stratification, enabling a more personalized therapeutic approach.

Below, we will report several prominent molecular classification models, focusing on those proposed in recent high-impact studies, a summary of which is presented in Fig. 3.

The proliferation and inflammation subtypes

One of the earliest classification models for HCC divides it into Proliferation and Inflammation subtypes, based on the differential expression of genes associated with cell proliferation and immune response[94,95]. The Proliferation subtype is characterized by the overexpression of genes involved in cell cycle regulation and oncogenic pathways, particularly Wnt/β-catenin and MYC signaling. This subtype is associated with a poor prognosis, including aggressive tumor growth and a high likelihood of vascular invasion[93]. The Inflammation subtype, by contrast, displays overexpression of immune-related genes, including those involved in interferon signaling pathways, which correlate with a relatively favorable prognosis. This subtype's immune-enriched microenvironment makes it potentially responsive to immune-modulating therapies, particularly checkpoint inhibitors targeting PD-1 and CTLA-4[96].

For the Proliferation subtype, targeted therapies aimed at inhibiting the Wnt/β-catenin and MYC pathways are under investigation. Small molecule inhibitors targeting MYC and Wnt/β-catenin are being explored, though challenges in drug development for these targets still remain[97]. The potential of the Inflammation subtype for immune checkpoint therapy has spurred clinical trials investigating PD-1 inhibitors, such as pembrolizumab, which has demonstrated promising results in HCC patients with immune-enriched tumor environments[98].

The Cancer Genome Atlas (TCGA) Classification: S1-S4 Subtypes

The Cancer Genome Atlas (TCGA) provided a comprehensive framework for classifying HCC into four molecular subtypes, known as S1-S4. This classification system offers deeper insights into the underlying genomic and epigenomic landscape of HCC tumors[99]. Each subtype is characterized by distinct gene expression profiles, reflecting different molecular pathways and clinical behaviors:

- S1 subtype: This subtype is characterized by activation of the Wnt/β-catenin pathway and metabolic dysregulation, often with a favorable prognosis due to its association with less aggressive clinical features.
- S2 subtype: Marked by TP53 mutations and chromosomal instability, S2 is associated with high genomic instability and poor outcomes. TP53 mutations in this subtype often lead to resistance to certain chemotherapies, necessitating alternative approaches.
- S3 subtype: Characterized by Ras/MAPK signaling activation, this subtype exhibits frequent growth factor receptor mutations, which can be targeted by tyrosine kinase inhibitors.
- S4 subtype: Featuring an immune-rich microenvironment, S4 shows high expression of immune checkpoint molecules such as PD-1 and CTLA-4, and is potentially responsive to immunotherapy, offering a more favorable outlook for patients with advanced HCC.

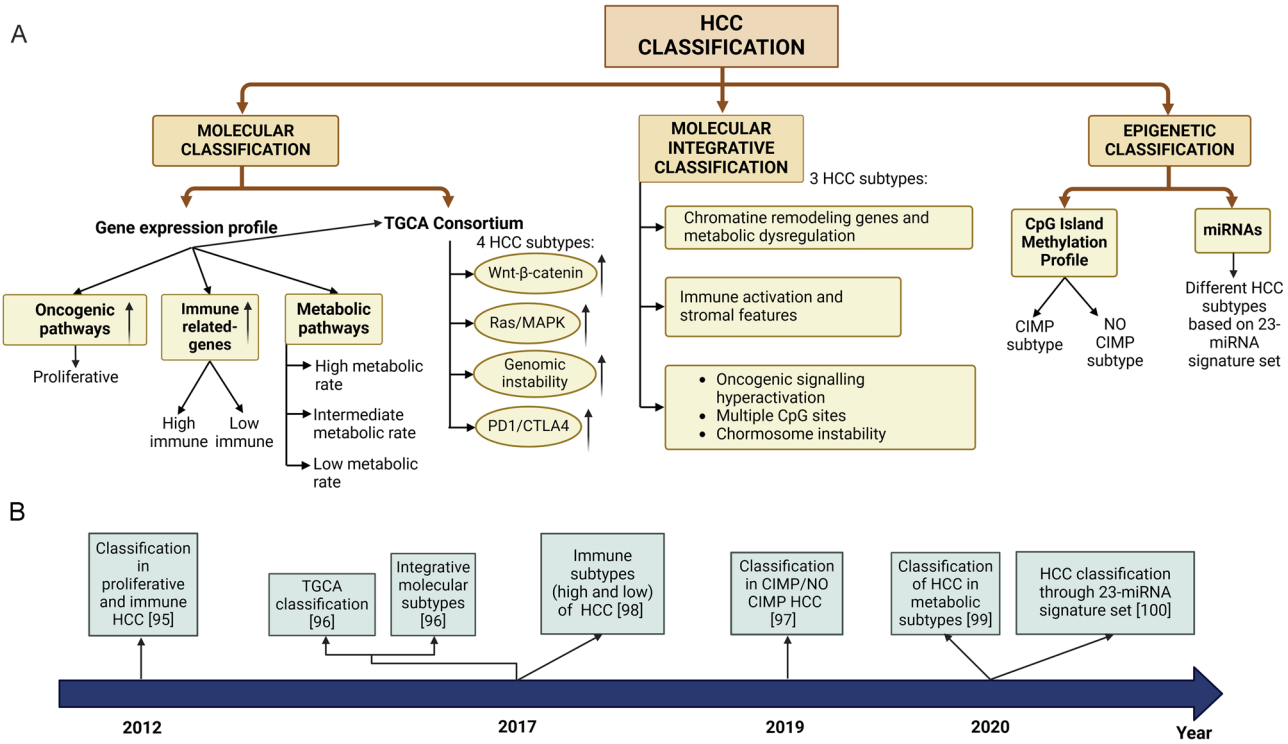

Fig. 3 | Molecular and genetic classification frameworks for hepatocellular Carcinoma. A Overview of the principal molecular and genetic classification systems proposed for HCC, with emphasis on their defining criteria and conceptual focus. **B** Timeline outlining the historical emergence and origin of each classification framework included in this study. Created with BioRender.com (https://BioRender.com/fdrtpgo).

The S4 subtype's immune signature makes it an ideal candidate for checkpoint inhibitors, which have shown significant promise in recent clinical trials. Conversely, S2 and S3 subtypes may benefit from therapies that address chromosomal instability and receptor-mediated pathways, such as tyrosine kinase inhibitors targeting growth factor receptors in S3[99].

iCluster subtypes: integrative molecular subtypes

Wheeler and Roberts introduced an integrative molecular approach through iCluster analysis, which combines gene expression data with epigenetic and proteomic information. This integrative classification is especially beneficial in cases where single-layer analysis (e.g., genomics alone) may not capture the full spectrum of tumor biology[99]. This approach revealed three main subtypes of HCC:

- iCluster 1: Enriched for chromatin remodeling genes and metabolic dysregulation, with a poor prognosis. This cluster showed a low frequency of cyclin-dependent kinase inhibitor 2 A (CDKN2A) silencing, mutation in CTNNB1 and TERT promoter, a reduced TERT expression, and, simultaneously, a high proliferation marker genes expression.
- iCluster 2: Marked by immune activation and stromal features, this group has a relatively favorable prognosis and may benefit from immunotherapy. It was characterized by lower-grade tumors with less microvascular invasion, suggest that these tumors may respond well to immunotherapy.
- iCluster 3: This group shows high activation of oncogenic signaling pathways, including Wnt/β-catenin and TGF-β pathways, chromosome instability, multiple CpG sites, and is associated with aggressive tumor characteristics and poor survival.

iCluster 1's chromatin remodeling features may be targeted with drugs designed to modulate epigenetic pathways. Meanwhile, iCluster 2 could benefit from immune-based therapies due to its immune-rich characteristics. iCluster 3, with its reliance on oncogenic signaling, may respond to TGF-β inhibitors or combination regimens targeting multiple pathways.

CIMP and non-CIMP subtypes

Li et al. categorized HCC based on the CpG island methylator phenotype (CIMP), a distinct epigenetic signature associated with hypermethylation of CpG islands in gene promoter regions[100]. The CIMP subtype is characterized by extensive DNA methylation and poor prognosis. This epigenetic subtype (CIMP subtype) is gaining attention as a potential biomarker for identifying patients who may benefit from novel epigenetic therapies targeting DNA methylation and chromatin remodeling enzymes.

The hypermethylation patterns in CIMP make this subtype a candidate for drugs like DNA methyltransferase inhibitors. Ongoing trials are exploring these drugs for HCC, providing a potential novel therapeutic option for patients with aggressive epigenetic profiles.

Immune subtypes

In addition to the Inflammation subtype described by Llovet et al., another classification by Sia and collaborators focused on immune-specific subtypes[96]. Using the potential of omics technologies, in particular the sequencing of the whole RNA of a tissue, they divided HCC patients according to different TME characteristics and they identified a novel "Immune class," comprising approximately 25% of cases, which exhibits heightened immune cell infiltration, elevated expression of immune regulatory molecules, and markers indicative of cytotoxic T-cell activity. This Immune class is further divided into two distinct subclasses based on immune microenvironment characteristics: an Active Immune Response subtype, associated with a favorable prognosis and enriched in adaptive immune signaling, and an Exhausted Immune Response subtype, marked by poor prognosis due to immunosuppressive signaling and T-cell exhaustion[96].

The Exhausted Immune subtype demonstrated upregulation of pathways associated with immune tolerance, including TGF-β signaling, which drives T-cell exhaustion and M2 macrophage polarization, potentially promoting tumor progression. Moreover, this subgroup exhibited high glycolytic activity and metabolic reprogramming, reinforcing its immunosuppressive phenotype. The findings suggest that patients within this

subtype may benefit from combination therapies targeting immune checkpoints alongside TGF-β inhibitors, potentially restoring anti-tumor immunity by reversing T-cell exhaustion. In contrast, the Active Immune Response subtype showed enhanced interferon (IFN)-γ signaling and cytotoxic gene expression, suggesting a pre-existing adaptive immune response that may facilitate responsiveness to PD-1/PD-L1 blockade therapies. This subtype aligns with the immune profiles seen in other immunotherapy-responsive tumors, such as melanoma, reinforcing its potential as a target for checkpoint inhibition[96].

This immune classification not only deepens understanding of HCC's immune landscape but also offers a potential biomarker for stratifying patients in clinical settings.

Metabolic subtypes

Yang et al. described a classification based on metabolic gene expression profiles, revealing subtypes with distinct metabolic pathways[101]. They are divided into three subtypes (C1, C2, and C3). C1 (active) was characterized by enrichment in signatures related to amino acid, lipid, and drug metabolism, showing low AFP expression, early pathologic stages (I/II), and lower histologic grades (G1/G2), which correlated with a favorable prognosis. C2 (exhausted) lacked distinct metabolic signatures but exhibited high levels of immune and stromal cell infiltration. C1 showed a strong association with metabolic activity and favorable outcomes mirrored the features of non-proliferative HCCs. In contrast, C2 showed elevated immune infiltration, making it potentially responsive to immune checkpoint inhibitors and chemotherapy. C3, which had elevated AFP levels and a worse prognosis, showed reduced metabolic activity compared to C1 but was still more metabolically active than C2. C3 was particularly enriched in hormone and proteoglycan metabolism, indicating an intermediate state with poor prognosis.

Proteomic subtypes

In early 2024, Diao et al. focused their attention on the characterization of immune subtypes and metabolic process in early-stage HCC to refine immunotherapy strategies[102]. Advances in omics technologies allowed the classification of HCC into subtypes based on immune activity and metabolic reprogramming, aiding in understanding HCC's immune microenvironment. Utilizing proteomics, they identified three distinct immune subtypes (IM1, IM2, IM3), each with unique immune infiltration and metabolic profiles.

Researchers identified three immune subtypes:

- IM1: Characterized by low immune infiltration and a favorable prognosis.
- IM2: Displayed intermediate immune and metabolic features.
- IM3: Showed high immune infiltration, T-cell exhaustion, elevated glycolysis, reduced bile acid metabolism, and the poorest prognosis.

Authors emphasize the connection between metabolic reprogramming and the immune microenvironment in HCC, especially the role of increased glycolysis and bile acid dysregulation in IM3. Through this classification, the study suggests that IM3 patients may benefit most from immunotherapy[102]. By linking metabolic features to immune activity, the findings propose new therapeutic approaches for HCC, emphasizing the need for immune subtype-specific treatments to enhance immunotherapy outcomes.

miRNA-based subtypes

MicroRNAs (miRNAs) are short, non-coding RNA molecules that regulate gene expression post-transcriptionally. Sathipati and Ho identified distinct molecular subtypes of HCC based on miRNA expression profiles[103]. Using a support vector machine (SVM)-HCC model, the authors identified a 23-miRNA signature associated with both early and advanced stages of HCC. The 23-miRNA signature was prioritized based on MED scores, with miRNAs having higher MED scores contributing more significantly to prediction accuracy. Functional insights into these top-ranked miRNAs were gained through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses, revealing their involvement in various cancer-related and non-cancer pathways. While this miRNA signature holds promise for predicting HCC stages, the authors also examined co-expressed miRNAs to further explore potential miRNAs beyond the 23-signature set. This broader analysis could provide deeper insights into the overall impact of miRNA activity on HCC progression.

Integration with clinical staging

Emerging molecular and genetic classification frameworks offer a more detailed understanding of the heterogeneity of HCC and could complement and improve upon existing clinical staging systems, particularly Barcelona Clinic Liver Cancer (BCLC) algorithm[1], which is still fundamental to guiding therapeutic decisions. While the BCLC system uses tumor burden, liver function, and patient performance status to predict prognosis and allocate treatment, molecular subtyping provides an extra biological layer that could improve risk stratification and support personalized treatment. Integrating these molecular insights into BCLC staging could enable more nuanced prognostication and better-informed treatment selection. Finally, while current clinical guidelines increasingly acknowledge the relevance of this translational approach, prospective validation studies are still needed to standardize the use of molecular classifiers in routine clinical practice.

Conclusions and future direction

HCC remains a tumor whose early diagnosis and prognosis are complex and often suboptimal using the markers currently described in guidelines. In our review, we focused on the critical need for, and recent advancements in, novel biomarkers for these purposes in patients at risk of or affected by HCC. The development of such biomarkers is essential in routine clinical practice, playing a pivotal role in improving patient outcomes and reducing healthcare costs. Recent studies have explored promising candidates like DCLK1, GPC3, CD276, FGF19, INMT, MMP10, DKK1, OPN, and innovative approaches such as single-cell sequencing and liquid biopsy. However, it is crucial to acknowledge that HCC should be understood as a complex family of tumors, exhibiting diverse histological and mutational features. This inherent heterogeneity means that some proposed markers may be suitable for one subtype but not for another. Classification based on gene and molecular expression profiling has provided valuable insights into this biological diversity, significantly improving the understanding of HCC and offering a framework for targeted therapies. Nevertheless, recent molecular and genetic subclassifications further complicate the study and identification of broadly usable markers for diagnosing, monitoring HCC development, and assessing the efficacy of implemented therapies.

In conclusion, while emerging biomarkers and technologies hold considerable promise for enhancing HCC management, continued and focused research is essential. Future directions must involve not only validating these candidates but also improving accessibility for clinical applications. The path towards a genuine advancement in HCC treatment lies in the implementation of personalized medicine. This necessitates a cohesive strategy that integrates the use of highly efficient biomarkers with thorough analyses of the specific genetic and molecular (including metabolomic) classifications unique to each patient's HCC subtype. Such an integrated approach is paramount to tailoring therapies effectively and ultimately improving patient outcomes.

Data availability

No datasets were generated or analyzed during the current study.

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Competing interests

The authors declare no competing interests.

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