REVIEW ARTICLE

The role of molecular pathology in the precision diagnosis and subclassification of hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) remains a leading cause of cancer death worldwide despite recent advances in surveillance and therapeutic management. The outcomes for HCC patients remain poor, often as a result of late diagnosis or lack of effective treatments. Early detection and precise diagnosis are evidently crucial in improving the prognosis of HCC. However, HCC is a highly heterogeneous cancer with various clinical backgrounds and altered molecular pathways; these factors make its precise diagnosis more difficult. Approximately 25% of HCCs harbor actionable mutations, which are yet to be translated into clinical practice. In the era of precision medicine, molecular or genomic information are indispensable for HCC diagnosis and prognosis. Exploring genomic alterations has become a requirement for identifying the molecular subtypes of HCC. Recent studies have introduced molecular markers to help identify early HCC and to clarify its multistep process of carcinogenesis. The subclassification of tumors into proliferation class and nonproliferation class HCCs gives pointers to the HCC phenotype and facilitates the selection of appropriate treatments. In this review, we broadly summarize some of the latest insights into HCC subclassification from the perspective of molecular pathology. Immunohistochemistry-based subclassification allows improved characterization of HCC in daily clinical practice. Moreover, analysis of the immune microenvironment, intra-tumoral morphological heterogeneity, and imaging features gives additional information regarding the classification of HCC. Combinations of these approaches are expected to inform and advance the precision diagnosis and management of HCC.

Keywords: Hepatocellular carcinoma, molecular subclassification, molecular pathology, immune subtypes

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Abbreviations

Bmi-1, B lymphoma Mo-MLV insertion region 1 homolog; CAP2, Cyclase-associated protein 2; CK19, cytokeratin-19; CTNNB1, catenin B1; EMT, epithelial-mesenchymal transition ; EOB-MRI, gadoxetic acid-enhanced MRI; EpCAM, epithelial cell adhesion molecule; GPC3, glypican-3; GS, glutamine synthetase; HCC, hepatocellular carcinoma; HSP70, heat-shock protein 70; HGeHCC, high-grade early HCC; LDGN, low-grade dysplastic nodules; LGeHCC , low-grade early HCC; HGDN, high-grade dyspalstic nodule; ICIs, immune checkpoint inhibitors; LGR5, leucine-rich repeat containing G protein-coupled receptor 5; OATP1B3, anion transporting polypeptide 1B3; PD-1, programmed cell death 1; SALL4, Sal-like protein 4; TERT, telomerase reverse transcriptase; TPMs, TERT promoter mutations

INTRODUCTION

In 2020, liver cancer was the sixth most common cancer and the third leading cause of cancer-related deaths worldwide. Incidence rates and mortality are two to three times higher in men than in women, and liver cancer ranks fifth in terms of global incidence and second in terms of mortality for men. Hepatocellular carcinoma (HCC) is the most common form of liver cancers, accounting for ~90% of all liver cancers. It is still the most common cancer in East and South-East Asia and is the leading cause of cancer death in Mongolia, Thailand, Cambodia, Egypt, and Guatemala among both men and women.[1] A study in Indonesia showed that there had been no improvement in the median survival rate of HCC for patients diagnosed in 2013–2014 compared with those diagnosed in 1998–1999.[2]

Hepatocellular carcinoma develops in association with major risk factors such as chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), consumption of aflatoxin B1-contaminated food, heavy alcohol intake, and metabolic syndrome (type 2 diabetes, obesity, and non-alcoholic fatty liver disease).[3] Despite recent advances in the surveillance and therapeutic management of HCC, prognosis is still poor and the disease remains a global health challenge.[4] Although the background conditions vary from country to country, HCC itself has diverse predisposing factors and genetic susceptibilities. Moreover, hepatocarcinogenesis is known to be a multistep process in which many genetic signaling pathways are involved. These characteristics result in HCC being an extremely heterogeneous solid cancer; indeed, among the tumor types so far identified, HCC is one of the most highly heterogeneous.[5] In the era of targeted molecular treatments as promising therapies for advanced cancer, it is important to understand the detailed nature of the heterogeneous features of HCC.[3,6] Consequently, a comprehensive analysis of the morphologic, immunohistochemical, immune, and/or mutational status of HCC is crucial to enhance our understanding of hepatocarcinogenesis and to improve the clinical management of HCC.

Clinical pathological assessment of HCC is now possible based on its molecular landscape and having a precise diagnosis has led to improved therapeutic strategies.[7] Here, we discuss a broad range of molecular targets that have been (or could be) used to help define a more precise HCC diagnosis and to more accurately reflect its features based on pathological perspectives. The understanding of molecular features that underline HCC onset and progression is crucial for more efficacious management approaches in the future.

Molecular markers of early HCCs

Hepatocellular carcinoma is characterized by a clear multistage process of tumor development. Many cases of HCC develop in damaged livers related to chronic HBV or HCV infection. Histopathologically, HCC starts as premalignant dysplastic nodules (DN) and early HCC (eHCC) before it develops to progressed HCC.[8] The concept of eHCC, i.e., small nodules with indistinct margins, has been accepted internationally since 2009 and was officially adopted in the fourth edition of the World Health Organization (WHO) Classification of Tumors of the Digestive System in 2010.[9,10] However, the diagnosis of eHCC is often challenging because it generally lacks obvious histological atypia, its molecular mechanism is unclear, and it possesses quite heterogeneous clinical behavior. Nonetheless, recent advances in immunohistochemical analysis using various molecular markers have allowed us to carefully evaluate eHCCs.

The heat-shock protein 70 (*HSP70*) gene is a housekeeping gene that assists with a variety of vital intracellular chaperoning functions. Expression of HSP70 radually increases with the stepwise progression of hepatocarcinogenesis, but this is not observed in benign nodular lesions; this fact makes HSP70 a useful marker for eHCC and could clearly distinguish HCC from chronic hepatitis or cirrhosis.[11] The expression profile analyses of HSP70s from multiple databases such

as The Cancer Genome Atlas (TCGA) and ONCOMINE show a significant increase in HSP70s expression in HCC tissues.[12] Glypican-3 (GPC3), a member of the heparan sulfate proteoglycan family, is known to be upregulated in HCC but is not detected in normal liver tissues or benign liver lesions. The combination of GPC3 and alpha fetoprotein as markers for eHCC increases the sensitivity to 76% for early-stage tumors <3 cm in size.[13,14] Glutamine synthetase (GS) catalyzes the synthesis of glutamine, which is the major energy source for tumor cells. Expression of GS was found to increase in a stepwise manner from early to advanced HCC and promotes invasion of HCC cells through mediating the epithelial-mesenchymal transition (EMT).[15] A recent report showed that combination biomarkers significantly improved the sensitivity of the panel for eHCC detection and currently, the use of HSP70, GPC3, and GS is established as a useful marker combination for eHCC diagnosis.[16,17] This three-marker panel can increase the diagnostic accuracy of liver biopsies and was described in the fifth edition of the WHO Classification of Tumors of the Digestive System.[18]

Additional markers have also proven useful in the diagnosis of eHCC. We previously reported the upregulation of the polycomb group gene member, B-lymphoma Mo-MLV insertion region 1 (BMI-1), in early-stage HCC.[19] The signaling pathway of BMI-1 may allow cells to maintain their self-renewal ability and may thereby link to neoplastic proliferation by acting as a negative regulator of the INK4a/ARF locus that encodes two important tumor suppressor proteins in human cancer, p16 and p19. We observed positive expression of BMI-1 in HCC as high-intensity dot patterns within the nucleus that may reflect BMI-1 activity as a transcriptional repressor by regulating chromatin silencing.[8] Expression of BMI-1 was particularly observed in early and well-differentiated HCCs, but not in the surrounding liver tissue, a fact that should facilitate the identification eHCC. The role of BMI-1 in carcinogenesis has been reported for

many solid and non-solid cancers; moreover, BMI-1 was recently identified in association with the epithelial–mesenchymal transition in cancer cells.[20-23] Another study suggested that the forced expression of BMI-1 promotes the malignant transformation of hepatic progenitor cells, thereby providing a link to its "stemness" properties and neoplastic proliferation.[24]

Cyclase-associated protein 2 (CAP2) is also involved in the progression of HCC. Cyclase-associated protein 2 is a bifunctional protein in which the N-terminal domain binds to adenylyl cyclase and the C-terminal domain binds to monomeric actin. Expression of CAP2 is not seen in normal liver tissue but its expression increases during HCC progression from DN, through eHCC, to progressed HCC. Positive CAP2 expression was frequently seen in stromal invasion, a characteristic feature of eHCC, and high expression of CAP2 was correlated with poor prognosis of patients with HCC.[25] Indeed, we found that CAP2 was not only associated with the process of skeletal muscle development, but it was also significantly associated with tumor size, poor differentiation, portal vein invasion, and intrahepatic metastasis.[26] In the zebrafish, a vertebrate model, the silencing of CAP2 expression resulted in the development of a shorter body, which may have resulted from the downregulation of actin by CAP2. Expression of CAP2 was also found to be colocalized with actin in the leading edge of the lamellipodium overlap of HCC cells; moreover, migration assays showed that CAP2 knockdown reduced cell motility. These findings support the functional association of high CAP2 expression and explain how it could help promote invasive behavior in HCC cells. The role of CAP2 in tumor progression is not observed only in HCC: we found that CAP2 expression also increased stepwise during the progression of malignant melanoma and is upregulated in the aggressive histologic type of epithelial ovarian cancer.[27,28]

Extending the evaluation of detailed histological features, such as scirrhous components, with specific molecular markers,

such as HSP70, BMI-1, and CAP2, showed that small vaguely nodular lesions could be further subclassified as low-grade eHCC (LGeHCC), high-grade eHCC (HGeHCC), low-grade dysplastic nodules (LGDN), or high-grade dysplastic nodules (HGDN).[29] The investigation of 66 small vaguely nodular lesions showed that 20 nodules were DN and 46 nodules (69.8%) were eHCC. Among these 46 eHCCs, 18 nodules (39.1%) showed marked stromal invasion and/or the presence of a scirrhous component and were subclassified as HGeHCC, whereas the remaining 28 nodules (60.9%) did not show these kinds of features and were subclassified as LGeHCC. Positive immunohistochemical expression of BMI-1 was found at lower rates in LGDN but at higher rates in LGeHCC. The difference between them was significant and should prove useful to support differentiation between premalignant dysplastic nodules and eHCC. A stepwise increase in the immunohistochemical expression of CAP2 was also seen from LGDN to HGeHCC. A significant expression of CAP2 in HGeHCC may already indicate the malignant potential of HGeHCC nodules, and that the transitional stage to advanced HCC may already be present in HGeHCC; as a result, the re-evaluation of treatment strategies for eHCC may be needed.

Molecular subclassification of HCCs

Recent advances in molecular subclassification by gene expression analysis have facilitated a new understanding of the molecular landscape of HCC. During the past two decades, many studies have proposed HCC subclassifications based on the molecular features of the tumor. Molecular classification of HCC proposed by Boyault, and Hoshida are widely accepted.[30] Genome-wide transcriptome microarray analysis and quantitative reverse-transcription polymerase chain reaction data in a series of 120 HCCs and 3 hepatocellular adenomas by Boyault et al.[31] resulted in the proposal of six robust subgroups of HCC, termed G1–G6. Tumors classified in the G1–G3

subgroups were associated with high chromosomal instability compared to tumors in the G4–G6 subgroups. A previous study conducted by Hoshida et al.[32] identified a certain commonality between subclasses defined by a meta-analysis that encompassed 603 HCC patients from both Western and Eastern countries. They revealed three robust HCC subclasses (termed S1, S2, and S3) that could be associated with clinical parameters and distinct biological processes in hepatocarcinogenesis.[33] Subclasses S1 and S2 are associated with large tumors and poor histological differentiation. Characteristics found to be similar in the S1 and S2 subgroups, such as activation of the AKT pathway and frequent p53 mutations, were also found in Boyault's G1–G3 groups. The less-aggressive S3 subclass (which retains a hepatocyte-like phenotype) also resembles the G5 and G6 subgroups, showing associations with the Wnt/β-catenin (CTNNB1) signaling pathway.

These concepts of HCC subclassification are increasingly accepted worldwide, and many gene alterations in HCC now can be associated with molecular subclassifications. LGR5, also known as G-protein-coupled receptor 49 (GRP49), has been well established as a target of the Wnt/β-catenin signaling pathway, particularly in colorectal cancer.[34] We also found that LGR-5 is notably overexpressed in HCC carrying β-catenin mutations. Stable clones of LGR5-overexpressing HCC cells formed nodular tumors with a tightly aggregated morphology, whereas downregulation of LGR5 changed the cells into a loosely associated morphology resulting in enhanced infiltration and increased cell motility.[35] Our findings regarding LGR5 function in HCC cells seem to be an exemplar of Hoshida's S3 subclass and Boyault's G5–G6 subgroups.[36] The typical morphological and biological features of LGR5 expression in HCC may indicate a subset of the less-aggressive HCC phenotype. Taken together, these studies suggest that, despite highly variable clinical backgrounds and molecular heterogeneity, HCCs may share a similar gene-expression pattern. At present, data

based on genomic profiling studies show that HCCs can be roughly divided into two major molecular clusters, namely the proliferation class and the nonproliferation class. Patients with HCC features consistent with the proliferation class may have aggressive tumors, higher alpha fetoprotein levels, moderate/poor cell differentiation, and frequent vascular invasion. In contrast, the nonproliferation class is characterized by a less aggressive phenotype, better histologic differentiation, and lower alpha fetoprotein levels.[37,38]

Characterizing HCC based on its distinctive molecular and clinical features is a prerequisite for precision medicine because it provides more accurate classification and diagnosis which allow more effective treatment. Clinical trials for HCC have suggested that targeted agents have different efficacy in diverse populations because different molecular pathways are involved in different HCC patients.[39,40] However, performing genomic profiling for every HCC case is not straightforward in daily clinical practice.

Consequently, we have proposed an HCC subclassification based on immunohistochemical staining. Immunohistochemical analysis was carried out for panels of several molecular markers commonly used in HCC, and our findings indicated that HCCs could be broadly divided into three subclasses. The biliary/stem cell marker positive subclass (B/S subclass) is indicated by positive staining for cytokeratin 19 (CK19, KRT19), sal-like protein 4 (SALL4), or epithelial cell adhesion molecule (EpCAM); the Wnt/β-catenin signaling-related marker positive subclass (W/B subclass) is indicated by positive staining for β-catenin or glutamine synthetase (GS); and the negative subclass (-/- subclass) is indicated when all markers are negative.[41] Representative HCC cases associated with B/S or W/B subclass are shown in **Figure 1**. The B/S subclass can be further divided into two subgroups: CK19 and/or SALL4 positive (B/S1 subgroup) and EpCAM only positive (B/S2 subgroup). The B/S subclasses, particularly the B/S1 subgroup, exhibit poor tumor differentiation, increased frequency
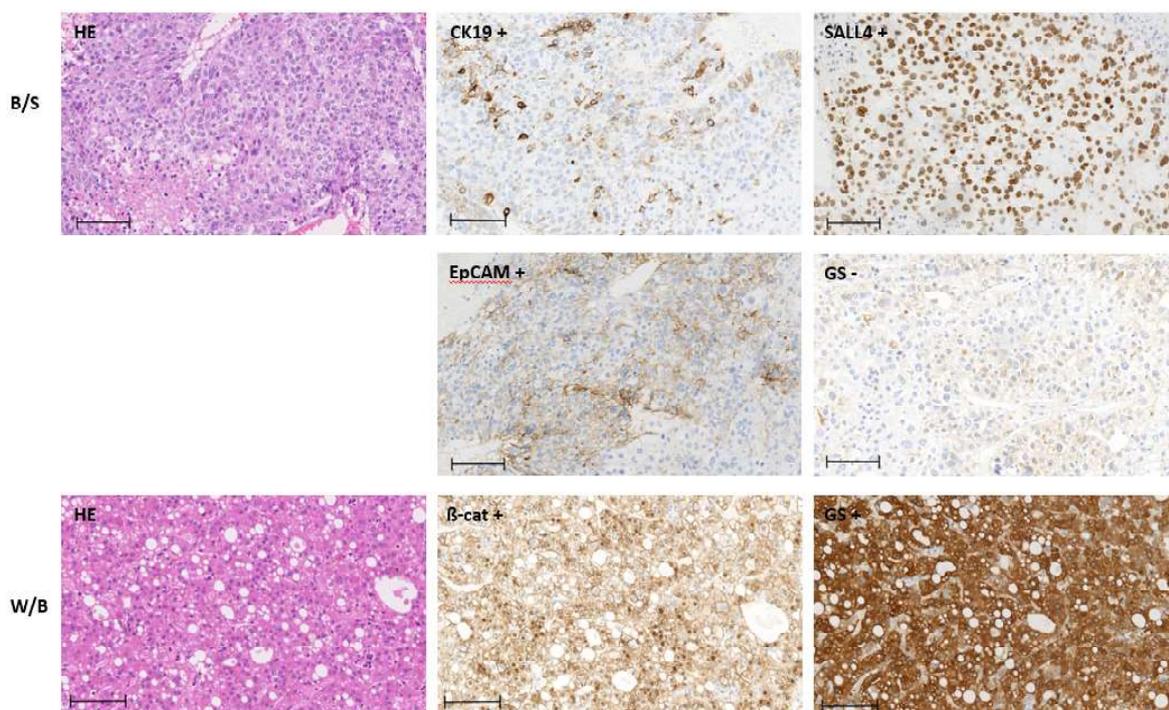

Figure 1. Representative HCC cases with positive immunohistochemical expression for CK19, SALL4, EpCAM, β-catenin, and GS. HCC case with CK19+, SALL4+, and EpCAM+ was included as B/S subclass (corresponds to negative GS staining); while HCC case with β-catenin+ and GS+ was included as W/B subclass. The two groups have been associated with different clinicopathological features. [41]
HE: hematoxylin and eosin staining. Scale bar = 100 μm.

of portal vein invasion and/or intrahepatic metastases, and highly proliferative activity. They are also associated with the shortest recurrence time. In contrast, the W/B subclass exhibits better tumor differentiation, a decreased frequency of portal vein invasion, and less proliferative activity.[41] These subclasses partly overlap with previous molecular subgroups from transcriptomic methods: the B/S subclass shares some features with the S2 and G1 subgroups, and the W/B subclass roughly corresponds to the S3 and G5-6 subgroups [7] (Table 1). The HCC phenotype was shown to be related to its molecular alterations and underlying oncogenic pathways.[42]

Furthermore, recent studies showed that cancer heterogeneity is also shaped by active immune responses. Immune cells are important elements of tumor tissues, and the amount of immune cell infiltration considerably differs among tumor types and histological subtypes. Most of the hallmarks of cancer are enabled and sustained to varying degrees by a tumor-supporting microenvironment.[43,44] Hepatocellular carcinoma is known to result from a complex interplay between genetic and environmental factors. Intervention in the immune microenvironment of HCC may benefit the management of HCC.[37,45] Recently, immune checkpoint inhibitors (ICIs) have shown potential as treatments for various cancers including HCC. A study showed that nivolumab, a programmed cell death protein-1 (PD-1) inhibitor, exhibited favorable efficacy and safety as a treatment for advanced HCC.[46] Nivolumab has since gained approval from the U.S. Food and Drug Administration for HCC patients previously treated with sorafenib.[47] Although the use of ICIs has been considered a promising approach to treat advanced HCCs, some patients have shown a limited response to such treatment. This again suggests that identifying HCC subclasses based on a combination of immune response and

Table 1. The concordance between different molecular subtyping methods for HCC

		Proliferation Class "More Aggressive Type"	Non-proliferation Class "Less Aggressive Type"	
HCC Tumor Cells	IHC-based Subclass	B/S -/-	W/B	(41)
	Molecular-based Subtypes	S2 S1	S3	(32, 33)
		G1 G2 G3	G4 G5-G6	(31)
	Associated Gene Signatures	KRT19, SALL4, EpCAM	LGR5	(70)
			OATP1B3, CTNNB1 mutation	
			TERT promoter mutation	(63)
Tumor Microenvironment	Immune Profile	High Mid Low	High Mid Low High Mid Low	(49)
	Response to Immunotherapy	Immune class (Responders to ICIs)	Immune exclusion class (Less response to ICIs)	(51, 52)
Clinicopathological Features	Etiology	HBV prevalence	HCV prevalence	
	AFP	high	low	
	Tumor differentiation	poorer	well to mod	
	Ki-67 index	high	low	
	Vascular Invasion	frequent	less	
	Outcome	worse	better	

Note: ICIs = immune checkpoint inhibitors; number in parentheses = reference number in body text

its association with clinicopathological findings is important for predicting prognosis and therapeutic efficacy.[45,48]

Our group showed that the immune microenvironment of HCC can be classified into three immune subtypes (namely, immune-high, immune-mid, and immune-low) that have additional prognostic impacts on histological and molecular classifications of HCC.[49] Comprehensive investigation of immune cells through multiplex immunohistochemical analysis of 919 regions of 158 HCCs indicated that increased B-cell, plasma-cell, and T-cell infiltrations were hallmarks of the immune-high HCC subtype. Immune-high-predominant HCC was found in nearly 20% of the immunohistochemistry-based B/S1 subclass and in less than 10% of the W/B subclass. Interestingly, the immune-high subtype indicates HCCs with better prognosis among poorly CK19-positive and/or SALL4-positive high-grade HCCs. However, the immunosubtypes were not prognostically significant in W/B HCC.[49] The immune-high subtype was also significantly enriched in Hoshida's S1 and Boyault's G2 subclasses, whereas the Immune-low subtype was observed in Hoshida's S3 and Boyault's G6 subclasses.[7] Recently, immune factors have been shown to be involved in the characterization of "cold tumor" phenotypes which are more resistant to immunotherapies.[50] HCCs with cold phenotype were associated with Wnt/β-catenin mutations and decreased infiltration of CD8+ T-cells into the tumor tissues.[51] This is a great challenge since it can promote immune escape and is less likely to respond to ICIs therapy. As described previously, around 30% of HCCs belong to the immune exclusion class and are associated with Hoshida's S3, Boyault's G5-6, and Kurebayashi's immune-low subtypes (Table1).[50-52] These results highlight the importance of comprehensive pathological evaluation of the immune microenvironment in addition to the standard histopathological and molecular classification of HCCs.

Intratumoral morphological heterogeneity in HCCs

The heterogeneous nature of HCC clearly represents a challenge to the establishment of a robust HCC classification system and HCC treatments. Intratumor heterogeneity is defined as distinct genetic alterations and phenotypes between cancer cells within the same tumor nodule.[53] Intratumor heterogeneity was reportedly detectable in most HCC cases (20 of 23, 87%), and heterogeneity solely at the level of morphology was found in 6 of 23 (26%) HCC cases.[6] The extent of intratumor heterogeneity varies considerably among HCC patients. Individuals with primary lesions larger than 5 cm showed a significantly higher extent of intratumor heterogeneity.[54,55] Intratumor heterogeneity can have major clinical consequences because the different genetic alterations and phenotypes between cancer cells, or between different tumor nodules, may require different treatment decisions.[53] Unlike many other cancers, HCC has not seen the benefit of individualized treatment due in part to intratumor heterogeneity.[54] Therefore, from the perspective of pathology, elucidating the relationship between morphological pattern and genetic alterations in HCCs is one way to gain further insight into hepatocarcinogenesis and to attain the goal of precision medicine.

Telomerase reverse transcriptase (TERT) is a catalytic subunit of the enzyme telomerase that is crucial for maintaining telomere elongation; furthermore, TERT *a*ctivation is considered to be a fundamental step in tumorigenesis. Mutation in the TERT promoter regions that could result in increased TERT expression have been identified in many cancers, including HCCs.[56,57] In HCCs, TERT promoter mutations (TPMs) reportedly occur at two hot spots: –124 base pairs (C228T), and –146 base pairs (C250T) upstream of the ATG translation start site. TPMs are also the most frequently found somatic genetic alteration in HCC, with an overall frequency of around 60%.[58-60] Interestingly, TPMs frequently occur early in the development of HCC and are highly

related to the stepwise process of hepatocarcinogenesis. From a series of 168 liver samples, TPMs were already identified in 6% of LGDN, 19% of HGDN, and 61% of eHCC; in contrast, other common gene mutations in HCC, such as those of CTNNB1 or TP53, occurred at a later stage.[61] These results show that TPMs are early events involved in the transformation of premalignant lesions to HCC; these findings also provide evidence that eHCC should get more attention as "HCC".[7,62]

Our group analyzed a total of 189 fresh-frozen liver tissue samples and found that 55% had TPMs.[63] In a retrospective analysis, all cross-sectional slides containing the whole tumor area were re-assessed for morphological histological patterns. The distribution percentage of each histological pattern in the tumor area for each HCC case was semi-quantitatively calculated using a homogeneity index. We found that HCCs with TPMs (TPM-positive HCCs) clearly exhibited intratumoral morphological heterogeneity as indicated by the smaller mean homogeneity index (0.800 ± 0.117 vs 0.927 ± 0.096, $p<0.0001$). TPM-positive HCCs displayed more diverse differentiation patterns, i.e., usually characterized by two or more histological differentiation patterns in each HCC nodule; in contrast, TPM-negative HCCs more often displayed single dominant patterns. Furthermore, early, or well-differentiated histological patterns were more commonly seen in TPM-positive HCCs than in TPM-negative HCCs [63] (**Figure 2**). We also noted that most of our HCCs with heterogeneous patterns had an HCV-related background and were likely to be TPM-positive. Previously, multiplex molecular profiling of HCC patients also revealed that patients with a *TERT* promoter mutation were more likely to be HCV positive rather than HBV positive and likely corresponded to Hoshida's S3 subtype (Table 1).[39,64] HCV-induced HCC development is thought to occur in a multistep process that involves the initiation of neoplastic clones accompanied by irreversible somatic genetic/epigenetic alterations and progression in
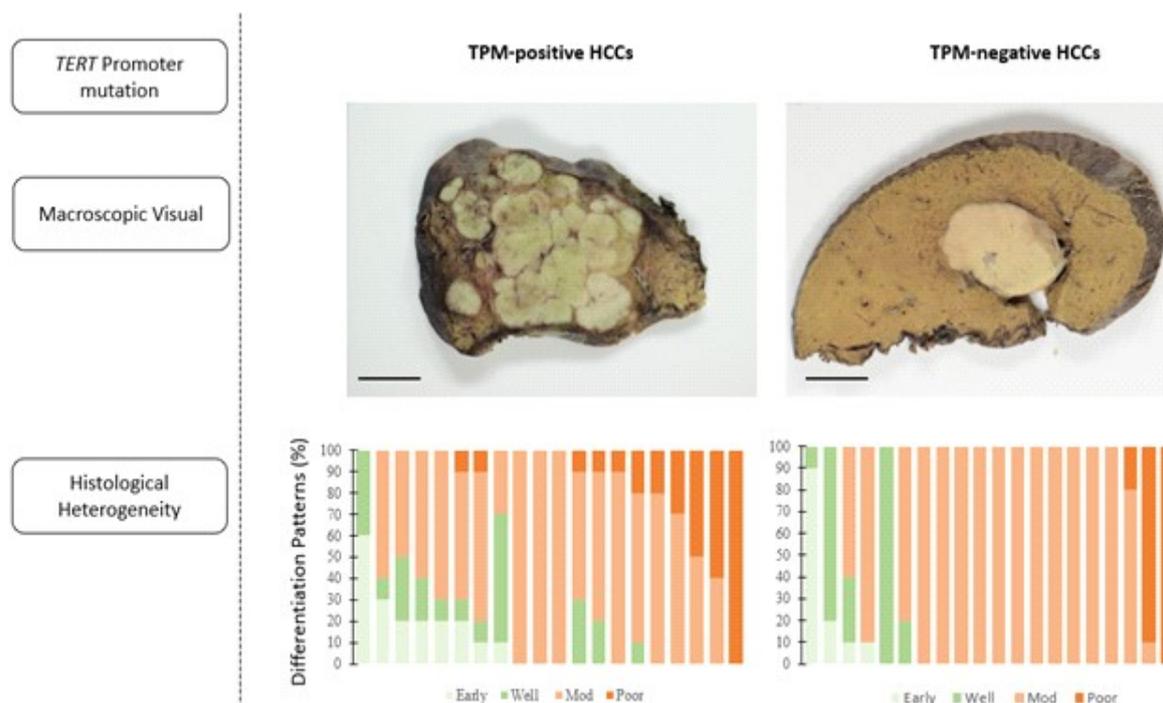

Figure 2. Intratumoral morphological heterogeneity based on the presence of TERT promoter mutations. Shown are representative gross specimens of a TERT promoter mutation (TPM)-positive HCC and a TPM-negative HCC. Histological differentiation patterns in the whole tumor area were evaluated as area percentages for each case of TPM-positive and negative HCC. TPM-positive HCC cases frequently displayed more variety in differentiation patterns than TPM-negative cases.[63] Scale bar = 100 μm

a carcinogenic tissue microenvironment.[65,66] Although additional genetic alterations appear to be required to develop clinically heterogeneous HCCs, TPMs may act as a precursor lesion in the early stage, as supported by our observation indicating a possible role of TPMs in HCCs with intratumoral heterogeneity.

The heterogeneous features of HCC might also be investigated through patho-radiological correlation studies. Several imaging modalities are commonly used for diagnosing HCC, such as ultrasound, computed tomography, and magnetic resonance imaging (MRI). Gadoxetic acid (synonymous with gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid, Gd-EOB-DTPA), a gadolinium-based MRI contrast agent, is a liver-specific (hepatobiliary) contrast agent that has expanded the role of MRI in simultaneously providing morphologic and functional information about the hepatobiliary system. Gadoxetic acid enters hepatocytes via active transport by organic anion transporting polypeptides (OATP1B1/3), and the resulting insights into the cellular mechanism of transportation have led to a better understanding of the correlation between radiologic and histologic features.[67,68]

Previous studies have shown that OATP1B3 expression could be important for evaluating the HCC tumor enhancement seen in gadoxetic acid-enhanced MRI (EOB-MRI).[69,70] We have observed that OATP1B3 expression decreases gradually during multistep hepatocarcinogenesis from the dysplastic nodule stage to progressed HCC. Moreover, different nodules in HCC may exhibit different features on EOB-MRI. HCC nodules with low intensity on the hepatobiliary-phase of EOB-MRI indicate a negative uptake of gadoxetic acid, whereas high-intensity nodules indicate a positive uptake. These nodules differentiated on EOB-MRI likely have different gene signaling alterations and HCC features. It has been reported that OATP1B3 is a downstream molecule of the Wnt/β-catenin signaling pathway,

and OATP1B3-upregulated HCC likely represents a specific subgroup of Wnt/β-catenin-activated HCC. [70] HCCs with mutations in CTNNB1 are likely to be well-differentiated tumors, as previously described in Boyault's G5 and G6 subclasses or Hoshida's S3 subclass. Indeed, HCCs with aggressive phenotypes are likely to have decreased OATP1B3 expression. CTNNB1-mutated HCC is likely associated with innate resistance to ICIs because it lacks inflammatory cell infiltration. Because recent studies have indicated that characterization of the immune microenvironment of HCC is important for predicting the effectiveness of treatments, performing EOB-MRI may be helpful in cases where immunotherapy is considered.[71,72]

Over the past few decades, considerable progress has been made in understanding the epidemiology, risk factors, and particularly the molecular features of HCC. However, the incidence rates and cancer-specific mortality of HCC continue to increase in many countries, and by 2025 more than 1 million individuals are predicted to be affected by HCC annually.[73-75] There is no doubt that continuous investigations on HCC heterogeneity features with comprehensive and integrative multidisciplinary approaches are still necessary to overcome HCC.

CONCLUSIONS

Hepatocellular carcinoma has variable molecular features that affect the diagnosis, treatment, and clinical outcome. Comprehensive analysis at the level of pathological features, including morphology, immune microenvironment, and genomic status, combined with imaging analysis is important to elucidate the heterogeneous features of HCC. Integrated analysis from pathological and molecular studies will help elucidate the development of hepatocarcinogenesis and facilitate precise diagnosis as the fundamental basis on which to develop the precision treatment of HCC.

CONFLICT OF INTEREST

There is no potential conflict of interest.

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CONTRIBUTORS

KE contributed to the writing and design of the manuscript, and drafting of the figures and tables. WTK drafted the figures. AU and MS reviewed the manuscript. All authors have read and approved the final manuscript.

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