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FRONTIER

Latest developments in precancerous lesions of hepatocellular carcinoma

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Abstract

Hepatocarcinogenesis in human chronic liver diseases is a multi-step process in which hepatic precancerous lesions progress into early hepatocellular carcinoma (HCC) and progressed HCC, and the close surveillance and treatment of these lesions will help improve the survival rates of patients with HCC. The rapid development and extensive application of imaging technology have facilitated the discovery of nodular lesions of ambiguous significance, such as dysplastic nodules. Further investigations showed that these nodules may be hepatic precancerous lesions, and they often appear in patients with liver cirrhosis. Although the morphology of these nodules is not sufficient to support a diagnosis of malignant tumor, these nodules are closely correlated with the occurrence of HCC, as indicated by long-term follow-up studies. In recent years, the rapid development and wide application of pathology, molecular genetics and imaging technology have elucidated the characteristics of precancerous lesions. Based on our extensive review of the relevant literature, this article focuses on evidence indicating that high-grade dysplastic nodules are more likely to transform into HCC than low-grade dysplastic nodules based on clinical, pathological, molecular genetic and radiological assessments. In addition, evidence supporting the precancerous nature of large cell change in hepatitis B virus-related HCC is discussed.

Key words: Hepatocellular carcinoma; Precancerous lesions; High-grade dysplastic nodule; Large cell change; Small cell change

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Core tip: The identification and characteristics of hepatic precancerous lesions may serve as early clues to malignant transformation. Over the last 10 years, studies of precancerous lesions have resulted in significant progress, especially in molecular biology and

imaging technology. Based on our extensive review of the relevant literature, this article focuses on evidence that supports the precancerous nature of dysplastic foci and dysplastic nodules from a clinical, pathological, molecular genetic and radiological point of view.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death around the world, and it is clinically characterized by a high incidence rate and poor prognosis. Despite the progress made in numerous treatments, the survival rate of HCC patients remains low because HCC is not easily detected prior to the advanced stage. Therefore, studies on the nature of precancerous lesions of HCC are important.

Hepatic precancerous lesions are currently recognized as nodular lesions that result from cirrhosis, and they are divided into two levels based on cytological and histological changes: microscopic dysplastic foci (DF) and macroscopic dysplastic nodules (DNs). DF are further classified as exhibiting large cell change (LCC) or small cell change (SCC)[1], the latter of which is widely observed in various precancerous lesions[2,3]. Specifically, molecular biology studies have shown SCC to be an intermediary stage between hepatocytes and malignant cells. DNs are further subdivided into low-grade dysplastic nodules (LGDNs) and high-grade dysplastic nodules (HGDNs) based on the degree of atypia[4], and the latter has a higher risk of malignant transformation.

Recent advances in pathology, molecular biology, genetics, and radiology have tremendously improved our understanding of hepatic precancerous lesions. Based on our extensive review of the relevant literature, this article focuses on the evidence showing that HGDNs are more likely to transform into HCC than LGDNs based on clinical, pathological, molecular genetic and radiological assessments.

DF

DF are microscopic lesions with an arbitrary diameter less than 1 mm, and they usually occur in a background of cirrhosis or chronic hepatitis[4]. DF often exhibit LCC (Figure 1A) or SCC (Figure 1B). According to a recent study[5], LCC is defined as hepatocytes displaying enlarged nuclei or cytoplasms that preserve their nuclear-cytoplasmic ratio, whereas SCC is defined as hepatocytes that exhibit decreased cytoplasmic volume, cytoplasmic basophilia, mild nuclear pleomorphism, hyperchromasia, and an increased nuclear-cytoplasmic ratio. Consequently, lesions with SCC are characterized by crowded nuclei and an increased cellular density.

LCC

LCC is currently thought to be a degenerative senescent change that results from chronic liver injury, but it could also be regarded as a predictive marker associated with HCC rather than a genuine premalignant lesion[6,7]. Conversely, LCC may actually be an important risk factor for HCC based on the following findings: (1) The patients with chronic hepatitis B exhibiting LCC were overall much more likely to develop HCC than patients without LCC[8]; (2) In most cases of human B viral chronic hepatitis/cirrhosis, hepatocytes exhibiting LCC had higher levels of proliferating cell nuclear antigen (PCNA)-labelling index (LI) and lower levels of transferase-mediated dUTP-biotin nick end labeling (TUNEL)-LI than cells without LCC[9], which suggests that HBV-related LCC is actively involved in hepatocarcinogenesis; (3) Abnormal DNA content (aneuploidy) is common in LCC, but this abnormality does not significantly differ from that observed in SCC[10]; (4) LCC and related molecular changes have been identified in a subset of patients infected with HBV, which suggests a direct premalignancy[11]; and (5) Compared with cholestatic LCC, HBV-related LCC exhibits significantly higher Tp53-LI, gamma-H2AX-LI and micronuclei index as well as shorter telomere length, decreased SA-beta-Gal activity and increased net cellular gain[12].

LCC is frequently detected in chronic hepatitis B cases with advanced histologic stage, particularly those with HCC[6]. Taken together, these findings indicate that LCC may not only be related to hepatocarcinogenesis but may be a precancerous lesion of HBV-related HCC.

SCC

SCC has long been thought to be a characteristic of early precancerous lesions of HCC based on the following findings: (1) SCC has frequently been identified in HGDNs and well-differentiated HCC (W-HCC). Specifically, the largest nuclei have been observed in W-HCC, and the nuclear/cytoplasmic ratio is higher in HGDNs and W-HCC than in other nodular lesions. Moreover, hyperchromasia is markedly more pronounced in W-HCC than in other nodules[2]; (2) Mosaicism for loss of X chromosomal inactivation has been demonstrated in nodules of altered hepatocytes (NAH) exhibiting SCC, and an array-based comparative genomic hybridization (array-CGH) analysis revealed that some of the chromosomal abnormalities in NAH with SCC coincided with the abnormalities in HCC[3], suggesting that NAH exhibiting SCC could be precursors of HCC; and (3) SCC is characterized

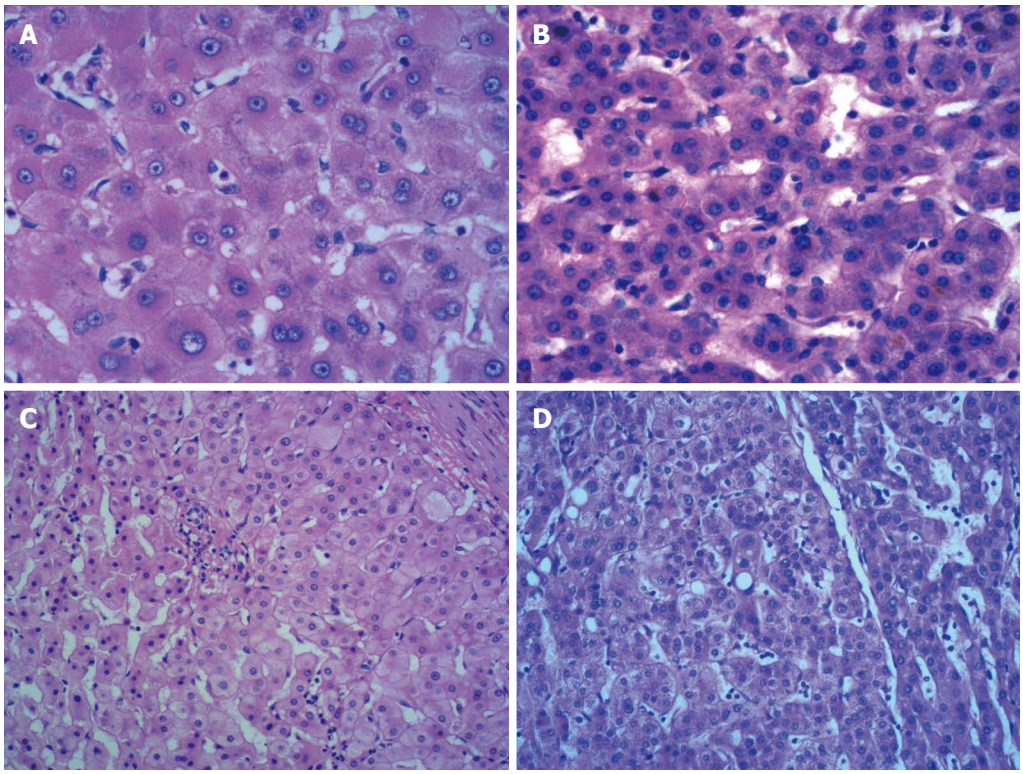

Figure 1 Histological characterization of large cell change (A), small cell change (B), low-grade dysplastic nodule (C) and high-grade dysplastic nodule (D). Large cell change is characterized by cellular and nuclear enlargement with preserved nuclear and cytoplasmic ratio, nuclear pleomorphism and hyperchromasia, and prominent nucleoli (A), and small cell change characterized by decreased cell volume, mild nuclear pleomorphism, an increased nuclear-cytoplasmic ratio, and increased nuclear density (B); Low-grade dysplastic nodule is characterized by minimal cytological atypia, slightly increased nuclear-cytoplasmic ratio and cell density (C), and high-grade dysplastic nodule characterized by high cell density, an increased nuclear-cytoplasmic ratio, hepatocytes organized in trabeculae that are two to three cells thick, and mild nuclear atypia (D). A-D: Hematoxylin-eosin staining, magnification × 200.

by high proliferative activity[13]. Conversely, the cumulative probability of developing HCC did not differ between patients with SCC and those without SCC in one study[8], suggesting that SCC in chronic hepatitis B should not be considered a risk factor for HCC development.

Surprisingly, a crosscheck of the literature revealed inconsistent or contradictory conclusions, even from the same group of authors, between different studies of the same marker. In one study[14], the expression of p21 and p16 in HBV/HCV-related cirrhosis was preserved in LCC, decreased in SCC, and absent in HCC, whereas gammaH2AX-DNA-damage foci were absent in LCC but present in SCC and in HCC. These data suggest that SCC occurs in more advanced precancerous lesions than LCC. In another study[12], the same group of authors found that p21 and p16 were activated in normal-looking cirrhotic hepatocytes (NLCH) of HBV-related cirrhosis and that this expression gradually diminished from LCC to SCC and HCC. They also reported that the expression of gamma-H2AX foci significantly increased during carcinogenesis, from normal hepatocytes to NLCH, LCC, SCC, and HCC. Thus, these data contradict the previous findings and suggest that HBV-related LCC may represent dysplastic hepatocytes rather than reactive hepatocytes. These conflicting findings may be attributed to the use of different racial and regional groups, *i.e.*, HBV (17 cases)/HCV (6 cases)-related cirrhosis samples in the former study and HBV-related cirrhosis samples in the latter study.

The nature of LCC and SCC as characteristics of true precancerous lesions and their relationship to HCC are fiercely debated[1]. Future studies should consider the following factors, which at least partly explain the inconsistency, if not conflict, in previous findings: SCC is a relatively rare finding that could easily be confused with liver regenerative cells. In some studies, the diagnosis of SCC was confirmed by fewer than two pathologists or the authors failed to give a definition and/or example of DF[15,16]. Therefore, SCC is not easily distinguished from liver regenerative cells based on current findings, which may limit the predictive value of SCC for HCC development[17]. Discrepancies in studies may also be due to differences in research methodology and regional groups under study.

Based on current studies, SCC may be a more advanced precancerous lesion than LCC, and LCC may be a very early precancerous lesion of HBV-related HCC. Accordingly, the identification of SCC and LCC in liver biopsies might be related to an increased risk of HCC over time, and such lesions warrant inclusion in pathology reports[18].

Table 1 Summary of the main pathological, molecular genetic and radiological features of distinction between low- and high-grade dysplastic nodules

	LGDNs	HGDNs	e/WD-HCC	Quoted literature examples
Cytologic features				
Small cell change	-	+	+	Roncalli *et al*[30]
Large cell change	±	±	-	Roncalli *et al*[30]
Architectural features				
Nuclear/cyto-plasmic ratio	±	+	+	Chang *et al*[2]
Increased cell density compared with surroundings	-	1.3 to 2 times	> 2 times or more	Park *et al*[4]
Pseudoglands	-	±	+	Nascimento *et al*[31]
Unpaired arteries	±	±	+	Park *et al*[4]
Portal tract	-	+	+	Kojiro *et al*[32]
Hyperchromasia/nuclear atypia	-	+	+	Nascimento *et al*[31]
Radiological features				
Hepatobiliary phase images	No hypointensity	Hypointensity (70%)	Hypointensity (97.5%)	Gatto *et al*[40]
Arterial phase	Iso/hyperintensity (100%)	Iso/hypointense (96.7%)	Iso/hypointense (27.5%)	Gatto *et al*[40]
Washout and arterial enhancement	No washout or arterial enhancement on CT or MRI	6 (6/6)showed arterial enhancement on CT, 4 (4/6) were hypervascular on MRI; 5 (5/6) on CT and 4 (4/6) on MRI	51 (51/74) were hypervascular on CT, 57 (57/74) were hypervascular on MRI; washout was not showed	Serste *et al*[25]
Molecular genetic biomarkers				
Overall FAL index	0.16 ± 0.07	0.33 ± 0.21	0.40 ± 0.23	Lee *et al*[27]
Chromosomal changes	Deletions and gains were not found	Deletions of 8p and gains of 1q	Deletions of 8p and gains of 1q	Tornillo *et al*[45]
TERT promoter mutations	6%	19%	61%	Nault *et al*[50]
TRF length	7.2 ± 1.97	4.0 ± 0.89	4.5 ± 0.85	Oh *et al*[53]
Telomerase activity	0.9 ± 0.56	1.7 ± 0.75	2.3 ± 1.39	Oh *et al*[53]
miRNAs (miR-224)	-	+ (50%)	+ (100%)	Gao *et al*[57]
DNA methylation Nuclear expression of Dnmt3a	-	+ (29.2%)	+ (53.8%)	Choi *et al*[62]
Immunohistological biomarkers				
VEGF	± (100%)	+ (100%)	+ (100%)	Park *et al*[67]
GPC3	± (5.48%)	+ (50%)	+ (57.1%)	Gong *et al*[1]
MN index (number of micronuclei per 3000 hepatocytes)	0.9 ± 0.16	3.1 ± 0.38	4.0 ± 0.58	Lee *et al*[77]

-: absent; ±: may be present; +: usually present. CT: Computed tomography; MRI: Magnetic resonance imaging; FAL: Fractional allelic loss; TERT: Telomerase reverse-transcriptase; TRF: Terminal restriction fragment; Dnmt3a: DNA methyltransferases 3a; VEGF: Vascular endothelial growth factor; GPC3: Glypican-3; MN: Micronucleus; LGDNs: Low-grade dysplastic nodules; HGDNs: High-grade dysplastic nodules; e/WD-HCC: Early/well-differentiated hepatocellular carcinoma.

DYSPLASTIC NODULES

Dysplastic nodules are usually found in chronic liver diseases. These lesions are primarily 1-1.5 cm in diameter and are classified as LGDNs (Figure 1C) or HGDNs (Figure 1D) based on the presence of cytologic and architectural atypia[19]. Pathologically, human HCC develops in the following multistep manner: from LGDNs to HGDNs, early HCC, W-HCC, nodule-in-nodule HCC, and finally, moderately differentiated HCC[20]. In recent years, the concept of multi-step human hepatocarcinogenesis has been well documented[21-23]. Advances in pathology and molecular genetics, as well as clinical follow-ups, have confirmed that DNs are precancerous lesions of HCC[4,24]. In particular, advances in medical imaging technology have provided a more intuitive understanding of the characteristics of DNs by facilitating the detection of such small nodular lesions.

For many years, HGDNs have been thought to be more closely related to HCC than LGDNs based on histopathological features and clinical follow-up studies[2,25,26]. In recent years, advances in pathology, molecular genetics, and imaging have further confirmed that HGDNs are more likely the advanced precursors of HCC than LGDNs[2,27]. The main pathological, molecular genetic and radiological features of distinction between LGDNs and HGDNs are summarized in Table 1.

CLINICAL FOLLOW-UP STUDIES

Clinically, LGDNs are considered precancerous lesions associated with a slightly elevated risk of malignant transformation, whereas HGDNs are considered advanced precursors of HCC associated with a high risk of transformation[4,28]. Moreover, HCCs have been demonstrated to occur more frequently in HGDNs than LGDNs[29].

Clinical follow-up studies have revealed that HGDNs are the most advanced precancerous lesions of the liver, with a risk of malignant transformation of approximately 30%-40% at 24 mo[24]. In another study, a total of 147 patients with non-malignant liver nodules were

followed over a median duration of 29 mo, and the HCC development rate was higher in patients with HGDNs than in patients with LGDNs[26]. Based on a multivariate analysis of histologic diagnosis and decreases in portal flow on computed tomographic arterial portography (CT-AP), the authors classified hepatic nodules into three groups: HGDNs, LGDNs, and regenerative nodules (RNs). The progression rate of HCC from HGDNs was high, and the annual HCC development rate exceeded 30% in the first 2 years. HCC developed more often from HGDNs than from LGDNs and RNs[28]. Therefore, HGDNs are true precancerous lesions of HCC.

HISTOPATHOLOGIC FEATURES

Compared with HGDNs, LGDNs lack appreciable cytologic and architectural atypia based on the following findings: (1) LCC is frequently observed inside and outside of LGDNs as microscopic (< 1 mm) DF. SCC is most frequently seen inside HGDNs[30]; (2) The nuclear/cytoplasmic ratio is higher in HGDNs and W-HCC than in LGDNs[2]; (3) HGDNs and HCC exhibit more pronounced basophilia in the cytoplasm than large regenerative nodules (LRNs) and LGDNs[31]; (4) Hyperchromasia and nuclear atypia are observed in HGDNs and HCC[31]; (5) A pseudoacinar pattern (or acinar formation) and thickened trabeculae are never seen in LGDNs but frequently appear in HGDNs and HCC[31]; and (6) The number of portal tracts is normal in LGDNs but reduced in HGDNs and early HCCs[32]. In addition, some HGDNs often contain one or more microscopic foci of W-HCC, whereas nodule-in-nodule lesions are absent in LGDNs, which suggests that HGDNs might be precancerous lesions of HCC[20,33,34]. In conclusion, these histopathologic features indicate that HGDNs represent borderline lesions and closely resemble W-HCCs[35].

IMAGING DIFFERENCES BETWEEN HGDNs AND LGDNs

Angiogenesis

Angiogenesis, such as sinusoidal capillarization and unpaired arteries, gradually increases during hepatocarcinogenesis from HGDNs to classic hypervascular HCC[36]. Compared with LGDNs, HGDNs and W-HCC contain more aberrant unpaired arterioles and capillarized sinusoids[25].

Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging findings

Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) has been shown to improve the detection and characterization of focal liver lesions. Specifically, this approach can differentiate LGDNs from pre-malignant HGDNs and early HCC[37].

The hypointense appearance in hepatobiliary phase is often considered a radiological marker of nodule differentiation[38]. The Gd-EOB-DTPA-enhanced MRI findings of nodules (LGDNs, HGDNs, and HCCs), which were histologically identified on cirrhotic and explanted livers, indicated nodular hypointensity at the hepatobiliary (HB) phase in 39/40 HCCs and 21/30 HGDNs but in none of the LGDNs, despite a lack of significant differences among the enhancement ratios (ERs) of HGDNs and HCCs at the HB phase[39]. Another study examined the ability of delayed phase imaging (DPI) gadobenate dimeglumine-enhanced MRI in addition to dynamic postcontrast imaging to improve the characterization of small HCCs, HGDNs and LGDNs. This study identified significant qualitative and quantitative differences in the hypointensity on DPI between LGDNs and the group consisting of HGDNs and HCCs[40].

In one study, 62 of 215 nodules exhibited atypical radiological behavior (atypical nodules refer to a lack of hypervascularization in the arterial phase and/or absent hypovasculature in portal phase[38]), and 19 out of 20 HGDNs/early HCC nodules were found to be hypointense in the HB phase[41]. Most HGDNs (20/30) were reported to be iso/hypointense in the arterial phase and hypointense in the late phase, where all LGDNs were characterized by iso/hyperintensity in both the arterial and late phases. The ERs of lesions did not significantly quantitatively differ between hypovascular HCCs and HGDNs in the arterial phase or between hypointense HCCs and HGDNs in the HB phase[39].

In addition, patients with chronic liver disease showing a hypointense hypovascular nodule in the liver on hepatocyte-phase Gd-EOB-DTPA-enhanced MRI are at a high risk of developing HCC[42]. Therefore, HGDNs, which represent hypointense hypovascular nodules, could be precancerous lesions of HCC.

"Washout" and arterial enhancement of HGDNs and LGDNs on CT or MRI

In one study, washout was observed in 5 HGDNs (5/6) on CT and in 4 HGDNs (4/6) on MRI, whereas washout or arterial enhancement was not observed in patients with LGDNs[25]. In another study, the frequency of washout increased to 93.8% (45/48) in HCCs and 69.3% (9/13) in HGDNs in cirrhotic livers, and these incidences were higher than that of arterial enhancement[43]. These findings suggest that lesions exhibiting washout or arterial enhancement in the cirrhotic liver may be malignant nodules.

Furthermore, arterial hypervascularization with washout in subcentimeter hypointense nodules in the HB phase on Gd-EOB-DTPA-enhanced MRI in patients with chronic liver disease has been shown to strongly correlate with progression to hypervascular HCC[44].

Therefore, the imaging differences between HGDNs and LGDNs indicate that HGDNs are closely associated with progression to HCC and likely are more advanced precursors of HCC than LGDNs.

BIOMARKERS FOR THE DISCRIMINATION BETWEEN LGDNs AND HGDNs - MOLECULAR GENETIC BIOMARKERS

Allelic losses

Allelic losses in some regions were found to be correlated to clinicopathologic features and HCC progression[27]. The overall fractional allelic loss (FAL) index represents the frequency of allelic losses.

Genome-wide allelotyping has been adopted to systematically evaluate allelic changes during hepatitis B virus-associated hepatocarcinogenesis. Specifically, these studies found that the overall FAL index of HGDNs was significantly higher than that of LGDNs and was very close to that of HCCs[27]. This result indicates that HGDNs are genetically closer to HCC in terms of allelic losses, suggesting that their biological behavior likely resembles that of early HCC. Another group of researchers investigated the genetic differences between macroregenerative nodules (MRNs), LGDNs, and HGDNs *via* comparative genomic hybridization and found that although allelic losses of 8p and gains of 1q were observed in three out of six HGDNs, chromosomal imbalances between LGDNs and MRNs were absent[45]. In addition, the array CGH analysis revealed that HGDNs and HCCs exhibited a loss of heterozygosity at 5q13.2 and 8p23.1[46], which suggests that the genetic changes in HGDNs and HCCs are similar. Overall, these studies provide genetic evidence to support the idea that HGDNs are precancerous lesions.

Telomerase reverse-transcriptase

Telomerase reverse-transcriptase (TERT) promoter mutations are the most frequent somatic genetic alterations in human HCC arising from both cirrhotic and normal livers. They are major early events in tumorigenesis that occur at the precancerous stages in cirrhosis[47-49]. One study found that TERT promoter mutations were highly correlated with hepatocarcinogenesis: mutations were identified in 6% of LGDNs, 19% of HGDNs, 61% of early HCCs and 42% of small and progressive HCCs[50].

In addition, the human TERT (hTERT) mRNA levels, as measured by real-time quantitative RT-PCR, positively correlated with hepatocarcinogenesis, and a significant induction in the transition between LGDNs and HGDNs was observed. Most HGDNs strongly expressed hTERT mRNA at levels similar to those of HCCs[51].

Telomerase expression and the maintenance of a critical telomere length (TL) in cancer initiation indicate that telomere shortening and telomerase expression initiate cancer by inducing chromosomal instability[52]. Several studies have demonstrated that many HGDNs have shorter telomeres and a higher telomerase activity (TA) than LGDNs, and the TL and TA in HGDNs are similar to those of DNs with HCC foci and HCCs[53,54]. These results suggest that HGDNs are more similar to HCCs than LGDNs.

MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNA molecules that are thought to play an important role in the regulation of gene expression[55]. A growing body of evidence indicates that the deregulation of miRNAs plays a crucial role in hepatocarcinogenesis[56]. Specifically, some miRNAs, such as miR-145 and miR-199b, are downregulated in the progression from LGDNs to small HCC; miR-224 showed no expression in LGDNs, moderate expression in HGDNs, and strong expression in small HCCs[57,58]. These results suggest that LGDNs are early lesions in the spectrum of hepatocarcinogenesis and that HGDNs could be considered advanced precursors of HCC within this spectrum.

Changes in DNA methylation

Epigenetic changes are reversible and heritable and include changes in DNA methylation. Aberrant epigenetic modifications occur at the earliest stages of neoplastic transformation and are now believed to be essential players in cancer initiation and progression[59]. The aberrant DNA methylation of CpG islands is catalyzed by DNA methyltransferases (DNMTs). In human hepatocarcinogenesis, the expression levels of DNMT1, DNMT3a and DNMT3b mRNAs progressively increase from LGDNs to HGDNs, HCCs in DNs, and advanced HCCs[60], suggesting that DNMTs are involved in hepatocarcinogenesis and that HGDNs may be more advanced precancerous lesions than LGDNs.

DNMT3A: DNMT3A may play a role in hepatocellular carcinogenesis by regulating the expression of some tumor-suppressor genes, such as PTEN[61]. Specifically, nuclear immunoreactivity to Dnmt3a has not been detected in non-neoplastic livers or LGDNs but was observed in some HGDNs and HCCs[62].

Ras association domain family 1 isoform A: Ras association domain family 1 isoform A (RASSF1A) is a tumor suppressor that is methylated in many human cancers, including HCC[63]. As human hepatocarcinogenesis progresses from chronic hepatitis/cirrhosis to LGDNs, HGDNs, and finally, HCC, the expression of RASSF1A tends to gradually decrease[63]. Moreover, aberrant DNA methylation and increased DNMT expression have been demonstrated to be features of tumor cells[64]. Overall, the above-mentioned findings indicate that HGDNs share some features with HCC and are precancerous lesions of HCC.

BIOMARKERS FOR THE DISCRIMINATION BETWEEN LGDNs AND HGDNs - IMMUNOHISTOCHEMICAL BIOMARKERS

Vascular endothelial growth factor

Angiogenesis refers to the process of blood vessel formation, and all tumors require new blood vessels

to grow. Evidence from human studies suggests that angiogenesis is not necessarily a characteristic of an invasive tumor but may be an early event during the pre-malignant stages of cancer[65]. Angiogenesis may be stimulated by several regulators, among which vascular endothelial growth factor (VEGF) seems to be the most important one[66]. The expression of VEGF is higher in HGDNs and early HCCs than in LGDNs[67], which suggests that VEGF expression may be a good indicator of precancerous changes and may be useful to better understand and prevent the transformation of HGDNs to HCC.

Glypican-3

Glypican-3 (GPC3) is an oncofetal protein that plays a key role in growth factor signaling to regulate the proliferative activity of cancer cells[68]. Several recent studies have demonstrated high GPC3 protein expression in HCC but not in hepatic para-carcinomatous and cirrhotic tissues[69-72], indicating that GPC3 may be used as an immunohistochemistry marker to differentiate HCC from benign hepatocyte nodules[73]. To date, several studies have reported higher GPC3 protein expression in HGDNs than in LGDNs[1,74,75]. These results suggest that GPC3-positive DNs, especially GPC3-positive HGDNs, are indeed precancerous lesions of HCC[74].

Micronucleus

Chromosomal damage and the formation of a micronucleus (MN) are believed to play a significant role in the pathogenesis of many malignancies[76]. An MN is a small nucleus, and increases in the formation of MNs are usually regarded as an indicator of chromosomal damage. In one study, the micronuclei index was significantly increased in HGDNs compared with LGDNs. HCC exhibited the highest micronuclei index compared with those of HGDNs and DNs with HCC foci[77]. Therefore, the micronuclei index may transition from LGDNs to HGDNs, DNs with HCC foci and HCC, suggesting that HGDNs harbor more chromosome damage than LGDNs during hepatocarcinogenesis.

PROBLEMS AND PERSPECTIVES

For many years, the differentiation of dysplastic lesions, particularly HGDNs, from early HCC has been a challenge for clinicians, radiologists and pathologists. A variety of imaging modalities are currently used to evaluate patients with chronic liver disease and suspected HCC. Of these modalities, CT, MRI and contrast-enhanced ultrasound (CEUS) have largely replaced biopsy for the diagnosis of HCC[78]. However, all imaging techniques may fail to detect and diagnose small HCCs, particularly in the presence of cirrhosis[79]. The detection and diagnosis of small nodules, such as DNs, by imaging techniques are also very difficult.

DNs are generally hypovascular in the arterial phase, but they can sometimes be hypervascular without "washout" during the portal/late phases of CEUS[80]. Therefore, imaging findings on CEUS overlap between DNs and W-HCCs[81]. HCC or HGDNs can be noninvasively diagnosed if arterial enhancement and washout are found in a single dynamic imaging examination; however, these findings are frequently discordant on both CT and MRI[25]. CEUS can be effectively used as a useful problem-solving method by utilizing its unique advantages when CT and MRI are contraindicated or their results are indeterminate[82]. Similarly, nodules considered indeterminate after CEUS have been evaluated by contrast-enhanced CT or MRI for diagnosis[83,84]. Despite the above-mentioned merits of these imaging modalities, the differentiation between "early" HCC and an HGDN using only imaging techniques is sometimes difficult[80]. Moreover, a recent study recommends performing a biopsy rather than additional imaging when the first imaging is inconclusive[25].

DNs, especially HGDNs, are borderline lesions, and the biopsy of DNs is associated with a high rate of false-negative findings due to histological heterogeneity within the nodules[85]. Therefore, differentiating from HGDNs based on a small biopsy specimen may be difficult or nearly impossible[86]. Consequently, the identification of potential biomarkers that reliably detect or diagnose HGDNs or early HCC is urgently needed.

An increasing body of evidence suggests that epigenetic changes contribute to hepatocarcinogenesis. Therefore, DNA methylation and miRNAs have been proposed as promising biomarkers[87]. Specifically, changes in DNA methylation are ubiquitous in human cancer and have been shown to occur early during carcinogenesis. As such, these changes may serve as biomarkers for the detection of HGDNs or early HCC[88]. The frequency of aberrant promoter methylation has been confirmed to increase during the progression from precancerous lesion to HCC[89]. Therefore, DNA methylation in precancerous or early neoplastic stages may serve as a biomarker for screening patients with an increased risk for HCC and detecting early cancer. Moreover, the deregulation of miRNAs plays an important role in human carcinogenesis. For example, the down-regulation of miR-145 and miR-199b and up-regulation of miR-224 are frequently observed in pre-malignant DNs, and these changes persist throughout HCC development[57], suggesting that miRNA expression may serve as a biomarker for the detection of HGDNs or early HCC.

However, the above-mentioned biomarkers are tissue markers for detecting HGDNs or early HCC in liver biopsy specimens. In other words, a biomarker will be useful for screening or the early detection of cancer only if it can be detected in a noninvasive or minimally invasive fashion without tissue biopsy[88].

Increasing evidence has verified that DNA methylation and miRNAs in the blood or other bodily fluids may serve as valuable biomarkers for the detection of early

HCC[90]. Hopefully, DNA methylation or miRNAs in the blood will be translated into clinical use in the near future, which would facilitate the early diagnosis of at-risk patients and the accurate assessment of disease progression[87]. In addition, when combined with imaging contrast, blood biomarkers will significantly enhance our ability to diagnose HGDNs or early HCC and screen patients who are at carcinogenetic risk. These advances would allow patients to receive early treatment and ultimately improve survival.

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