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Review Article

Current and Emerging Molecular Markers of Liver Diseases: A Pathogenic Perspective

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Abstract

In the past decade, with the rapid development of molecular medicine and the application of more sophisticated methods for disease diagnosis and treatment, a number of molecular markers have become available for liver diseases. Pathogenesis-related markers are likely to be effectively discovered and rigorously validated, due to the unique biological links to diseases. The present study reviews the predominant clinical and research articles in the previous decade to provide a pathogenic perspective of current and emerging biomarkers for liver diseases, including hepatocellular neoplasms (*e.g.* hepatocellular carcinoma), non-neoplastic hepatocellular diseases, intrahepatic biliary diseases, and other liver diseases. Although it remains challenging to cover all markers for the diagnosis and prognosis of liver diseases, current and emerging molecular markers in clinical practice and under investigation were reviewed in a wide spectrum of liver diseases, in order to help clinicians and researchers identify liver disease markers for reference.

Introduction

Chronic liver disease and cirrhosis account for 44,000 deaths in the United States and two million deaths worldwide each year, and primary liver cancer was diagnosed in more than 40,000 adults in 2022 in the United States, as estimated by the American Society of Clinical Oncology.[1] This leads to the high burden of disability, and increases healthcare utilization. A number of traditional liver markers, including serologic and immunohistochemical markers, do not directly reflect the liver disease mechanism. Therefore, there is a need to identify better molecular markers for its diagnosis and prognosis. In the past decade, with the rapid progress of molecular medicine and the application of more sophisticated methods for disease diagnosis and treatment, a number of molecular markers have become available for liver diseases.[2–6] The present review provides a summary of current and emerging molecular markers for common liver diseases. Emerging proteomic and artificial intelligence tools can greatly help identify more sensitive, yet specific, markers.[7,8] However, a common challenge in developing molecular markers for liver diseases, as in other fields, is to determine how to effectively identify and rigorously validate these.[4] Pathogenesis-related markers may be the best leads for unique biological links to disease development, and these would likely provide a high-yield. Hence, the present review provides a pathogenic perspective on current and emerging biomarkers for liver diseases. It is noteworthy that molecular markers may be associated with and important for predicting the progression of some liver diseases. However, due to the limited space and scope of the present review, this topic was not discussed in length, despite its importance.

Keywords: Molecular; Marker; Liver; Pathogenic.

Abbreviations: AFP, alpha-fetoprotein; AIH, autoimmune hepatitis; AMAs, anti-mitochondrial autoantibodies; b-HCA, β-catenin-mutated type with the upregulation of GS; BAF, biliary adenofibroma; BDA, bile duct adenoma; cHCC-CCA, combined hepatocellular-cholangiocarcinoma; CK, cytokeratin; CRP, C-reactive protein; EHE, epithelioid hemangioendothelioma; EMA, epithelial membrane antigen; FNH, focal nodular hyperplasia; GS, glutamine synthetase; H-HCA, hepatocyte-nuclear-factor-1α mutated; HB, hepatoblastoma; HC, Hemochromatosis; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; IHCA, inflammatory type hepatocellular adenoma; ISH, in situ hybridization; MAFLD, metabolic-associated fatty liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; TERT, telomerase reverse transcriptase; ULC, undifferentiated liver carcinoma.

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Molecular markers for hepatocellular diseases

Malignant hepatocellular tumors

Hepatocellular carcinoma (HCC)

More than 90% of HCCs are correlated to a known etiology,[9] and

hepatocarcinogenic mechanisms can be classified as etiologically specific and nonspecific mechanisms.[10] Specific mechanisms include hepatitis B *via* viral integration, with the constant cis- and trans-activation of oncogenic factors,[11] hepatitis C *via* the oncogenic effects of the core antigen and NS5A protein,[12,13] and aflatoxin *via* direct genotoxic effects, leading to TP53 codon 249 mutations.[14] Nonspecific mechanisms accumulate the abnormalities imposed by chronic liver diseases.[15] HCC usually develops from chronic liver disease to a dysplastic nodule, prior to progression into HCC. The molecular markers for high-grade dysplasia include telomere shortening, telomerase reverse transcriptase (TERT) activation, and cell-cycle checkpoint inactivation.[16] Early HCC accumulate mutations in *CTNNB1*, which encodes β-catenin, and progressed HCC further presents with *TP53* mutations, DNA amplification, alterations in methylations, and other genetic abnormalities.[17] Multiple immunohistochemical markers are used to assist in the HCC diagnosis: polyclonal CEA, CD10, HepPar, arginase-1, and albumin *in situ* hybridization (ISH) are used as hepatocellular markers, while glypican-3, glutamine synthetase (GS), HSP70, CD34, alpha-fetoprotein (AFP) and clusterin are used to identify hepatocellular malignancy.[2] HSP70, glypican-3 and GS have been recommended in international guidelines.[18] Molecular testing is used for DNAJB1-PRKACA translocation, in order to diagnose fibrolamellar variant HCCs.[3]

Serologically, due to the low sensitivity (20%) in early HCC and fluctuating levels in cirrhosis, AFP was removed from the present screening assessment guidelines of the Canadian Association for the Study of the Liver (CASL), and the European Association for the Study of the Liver (EASL).[19,20] However, AFP is still presently used with other serological biomarkers, such as Lens culinaris-agglutinin-reactive fraction of AFP, and protein-induced by vitamin K absence or antagonist-II (PIVKA-II), for high risk populations.[21] Furthermore, studies have determined the des-gamma-carboxy prothrombin in patients with negative AFP. The results revealed that AFP was positive in 67% of HCCs, while AFP was negative in 66% of small HCCs and 20% of all HCCs.[22,23] However, none of the serologic markers were accepted by clinical practice guidelines for HCC screening due to cost-effectiveness, challenges in availability, and study result variations.[19]

Numerous markers are under investigation. Autophagy-related genes and its regulatory proteins are associated with HCC, including Beclin-1, ATG5 and ATG7, and these control a large number of molecular pathways in HCC oncogenesis, such as phosphatidylinositol-4,5-bisphosphate 3-kinase PI3K/AKT/mTOR, ERK/mitogen-activated protein kinase (MAPK), and apoptosis p53 pathways.[24,25] For example, the ATG-4B mRNA expression controlled by autophagy-related genes may contribute to HCC development *via* the noncoding of miRNA-661, and this has been proven to be clinically useful, with 100% sensitivity, in a clinical validation, especially in early-stage HCC.[26] Furthermore, HBV-related HCC is associated with mutated *TP53*, which involves the genetic integration with host genomes.[27] HCV-related HCC overexpress the Kinesin family member 20A, Cyclin B1, Hyaluronan-mediated motility receptor, and other genes. In addition, these markers are linked to lower survival in patients with HCV-associated HCC.[28] A study on IL-28 genetic polymorphisms revealed the association of the T allele with higher risk of HCC development.[29] Another study revealed that two genotypes of certain single nucleotide polymorphisms (SNPs) of IL-28 were associated with lower risk of HCC development.[30] Thus, the role of IL28 in diagnosing and prognosticating HCC appears unclear, if not contradictory.

Molecular factors are also used for the prognosis. Cytokeratin 19 (CK19) positivity is associated with increased recurrence rates, nodal metastasis, and more resistance to trans-arterial chemoembolization and percutaneous radiofrequency ablation.[31–33] The expression of miR-1180-3p increases in HCC, and is linked to tumor proliferation and poor survival.[34] A study conducted on KEGG pathways revealed that this epigenetic marker is associated with the regulation of the MAPK pathway, cell proliferation, apoptosis, and cell differentiation.[34]

Immune checkpoint proteins drive signaling pathways that suppress T-cell function,[35] including PD-1, PD-L1 and CTLA-4. Nivolumab was the first US Food and Drug Administration (FDA)-approved anti-PD-1 antibody for treating HCC. In addition, in 2020, the FDA granted the accelerated approval to nivolumab, in combination with ipilimumab, which targets CTLA-4 for the treatment of patients with HCC, who were previously treated with sorafenib.[36] Furthermore, a study has recommended the anti-PD-1 antibody agent for PD-L1 positive HCC patients.[37] Tumor mutation burden and microsatellite instability (MSI)/mismatch repair (MMR) are used to guide the immunotherapy for several cancers. These may play an important role in HCC immunotherapy in the future.[2]

Hepatoblastoma (HB)

Approximately 80% of HBs exhibit genetic alternations in the Wnt/β-Catenin signaling pathway. These alterations include the deletion of *CTNNB1* exon 3, *AXIN* genes, and the *APC* gene.[38–40] Overexpressed targets for Wnt signaling were also observed, such as cyclin D1, survivin and MYC. In addition, MYC further activates the Wnt signaling as a positive feedback mechanism.[41] The genomic profiling of HB can be classified into two subtypes, based on genetic instability (gains of chromasomes 8q and 2p): the overexpression of hepatic progenitor cell markers (AFP, CK19 and EpCAM), and the upregulation of MYC. Tumors with genetic instability are more aggressive, with a higher grade, and are more likely to metastasize.[42] Histopathologically, HBs can be classified as epithelial or mixed epithelial, and mesenchymal.[43] Epithelial HB may consist of fetal, embryonal, small cell undifferentiated, cholangioblastic and macrotrabecular components. β-catenin and glutamine GS are expressed in mesenchymal and fetal components.[44] Furthermore, AFP highlights less-differentiated epithelial components, and HepPar1 can be observed in more differentiated epithelial components. Moreover, glypican-3 is expressed in epithelial fetal and embryonal components.[15] In addition, CK7 and CK19 are positive in cholangioblastic components. SMARCB (INI1) highlights all HB components, except for small cell undifferentiated components.[15]

Benign hepatocellular tumors

Focal nodular hyperplasia (FNH)

The pathogenesis of FNH has not been fully explored. The presence of large vessels and vascular anomalies suggest the etiology of focally elevated blood flow.[45] Studies have revealed the altered expression of angioporitin genes, *ANGPT1* and *ANGPT2*, with an elevated ANGPT1:ANGPT2 ratio in FNH.[46] The activation of the β-catenin pathway would result in a "map-like" GS expression, without mutations in *CTNNB1* or *AXIN1*.[47,48] The immunohistochemistry for FNH revealed that LFABP retained its normal expression, β-catenin was negative for nuclear expression, and serum amyloid A and C-reactive protein (CRP) were usually negative.[6] Furthermore, patchy serum amyloid A or peri-septal CRP staining may be observed in some FNH cases.[49]

Hepatocellular adenoma (HCA)

HCAs are clonal benign neoplasms of four common subtypes: hepatocyte-nuclear-factor-1α mutated (H-HCA), β-catenin-mutated type with the upregulation of GS (b-HCA), inflammatory type (IHCA) with the serum-amyloid-A overexpression, and unclassified type.[50] H-HCA demonstrates biallelic HNF1A and CYP1B1 inactivation mutations. Liver fatty-acid binding protein is the characteristic for this group. IHCA activates IL-6/JAK/STAT due to mutations of the IL6ST gene, which codes gp130, FRK, STAT3, GNAS. and/or JAK1. C-reactive protein/serum amyloid A are usually diffuse positive, with a well-defined demarcation. The b-HCA- and β-catenin-activated IHCA (b-IHCA, having features of both IHCA and b-HCA) presents with CTNNB1-activated genomic abnormalities, leading to β-catenin pathway activation. Immunohistochemical marker GS is a good surrogate for genetic abnormality. GS diffuse homogeneous overexpression indicates the exon 3 mutation, GS heterogeneous staining with a starry-sky pattern indicates the exon 3 S45 mutation, and a GS faint expression indicates the exon 7/8 mutation. The exon 3 mutation is usually associated with high risk of HCC.[15]

The term, "borderline lesion" or "atypical hepatocellular neoplasm," has been used for b-HCA with cytologic atypia, but this remains insufficient for the diagnosis of HCC. This type has a high likelihood of HCC development. The *TERT* promoter mutation, as a typical genetic change in HCC, is usually identified in b-HCA/b-IHCA, with malignant transformations.[51,52] Since surgical resection is recommended for b-HCA/b-IHCA and borderline lesion, it is crucial for β-catenin activation to be detected for *CTNNB1* mutations. Molecular testing for *CTNNB1* genomic abnormalities, *TERT* promoter mutations, and chromosomal gains (1, 7 and 8) may be warranted when GS immunostaining is equivocal.[15]

In additional to the common HCA subtypes, sonic hedgehog HCA (shHCA) has been reported to present with somatic deletions of INHBE, leading to the fusion of INHBE and GLI1, and this special group may be identified by PTGDS immunostaining.[53] Argininosuccinate synthase 1 (ASS1) overexpression has been reported in another subtype of HCA (ASS1-positive HCA), and both subtypes are associated with high risk of hemorrhage.

Non-neoplastic hepatocellular diseases

Autoimmune hepatitis (AIH)

Autoimmune hepatitis (AIH) is an inflammatory liver disease in patients of all ages, and has female dominance. The key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies.[54] AIH type 1 can affect both adults and children, with characteristic positive anti-nuclear and/or anti-smooth muscle antibodies. On the other hand, AIH type 2 mostly affects children with characteristic positive anti-liver-kidney microsomal-1 and/or anti-liver cytosol-1 antibody. The autoantigens for type 2 AIH include cytochrome P4502D6 (CYP2D6)[55] and formiminotransferase cyclodeaminase (FTCD),[56] while those for type 1 AIH remain unclear. The genomic predisposition has been studied in AIH. Type 1 AIH presents with MHC class II HLA DRB1*03, which can be observed in all age groups, and DRB1*04, which is a late onset disease. Type 2 AIH presents with changes in DRB1*07 and DRB1*03.[57] It has been reported that the serologic parameters of lymphocyte-to-platelet ratio (LPR) and immunoglobulin-to-platelet ratio (IGPR) are independently linked to the liver fibrosis stage in AIH patients without prior treatment.[5]

Metabolic-associated fatty liver disease (MAFLD)

MAFLD, which was previously termed as, non-alcoholic fatty liver disease, is defined by the presence of >5% steatosis and metabolic risk factors, especially type-2 diabetes, obesity and metabolic syndrome, with the exclusion of excessive alcohol use.[58] The reasons for the interindividual variability may be attributable to the different genetic backgrounds, epigenetic modifications and epitranscriptomics, and these are the recently described biological determinants.[59] Genetic variants involved in liver lipid-metabolism are the major genetic risk factors for MAFLD, which include PNPLA3, TM6SF2, GCKR, MBOAT7, and HSD17B13.[60,61] Furthermore, epitranscriptomics is an emerging field, which helps understand how chemical RNAs and their modifications control RNA structures and functions, without changing the sequences. A large number (>100) of chemical RNA modifications have been described. Among these, N6-methyladenosine (m6 A) plays an important role in glucose and lipid homeostasis, and is involved in the progression of MAFLD.[62] In light of the gut-liver crosstalk, gut-specific PPARα may be applied as a novel target and predictive biomarker of NAFLD treatment.[63]

Hemochromatosis (HC)

HC is genetically heterogeneous, exhibits the uncontrolled iron absorption in the small intestine, and may present with progressive iron overload.[64] Its complications include arthritis, diabetes, heart failure, hepatic cirrhosis, and HCC.[65] Recent reviews and guidelines have classified HC into four types, based on its genotype-phenotype correlation, and type 2 and type 4 were further subdivided into subtypes A and B. The involved genes are, as follows: type 1, HFE; type 2a, HJV (hemojuvelin); type 2b, HAMP (hepcidin); type 3, TFR2 (transferrin receptor 2); type 4a and 4b, both SLC40a1 (ferroportin).[66,67] Although type 4a and 4b are associated with the same gene, the transferrin saturation (TSAT) in type 4a is usually low-to-normal, unlike the elevated TSAT in type 4b and other types. Liver biopsy is usually used to predict the disease progression and outcomes of patients with repeatedly high serum ferritin levels (>1,000 μg/L), and helps prevent and identify advanced fibrosis or subclinical cirrhosis before cirrhosis is developed. Indeed, the close surveillance for HCC is warranted, even for patients treated with iron depletion, when advanced fibrosis or subclinical cirrhosis is identified.[67]

Molecular markers of intrahepatic biliary diseases

Intrahepatic cholangiocarcinoma (iCCA)

iCCA is a malignant intrahepatic epithelial neoplasm with biliary differentiation, and expresses biliary markers, such as epithelial membrane antigen (EMA), CK7 and CK19. There are two subtypes of iCCA: large duct and small duct. Large duct iCCA may develop from biliary intraepithelial neoplasia or intraductal papillary neoplasm of the bile ducts,[68,69] while the carcinogenesis of small duct iCCA has not been fully explored. This may develop from liver progenitor cells,[70] or from transformed and transdifferentiated hepatic progenitor cells, or mature hepatocytes.[71,72] Due to the different cell origins of large duct and small duct iCCA, the expression of a number of markers differ between these two subtypes. Small duct iCCA is positive for CD56, C-reactive protein, N-cadherin and IDH1/2 mutations, while large duct iCCA is positive for MUC5AC, MUC6, S100, TTF1, AGR2, MMP7 and KRAS mutations.[73–75] Based on the integrative analysis of expression and mutation profiles, iCCA can be classified into proliferation and inflammation subclasses. The inflammation subclass presents with the activation of inflammatory pathways, the overexpression of cy-

tokine IL10/IL6, and STAT activation. The proliferation subclass presents with the activation of oncogene signaling pathways, with the positivity of RAS, MAPK, c-MET, BRAF and KRAS. The proliferation subclass genomically resembles poor-prognostic HCC.[15] The C-reactive protein expression in iCCA is associated with a better prognosis, while the EMA expression implies a worse prognosis.[71,76] Small duct iCCA has better overall survival and longer time to recurrence, when compared to large duct iCCA.[77]

Benign biliary tumors

Bile duct adenoma (BDA)

The pathogenesis of BDA remains controversial. It has been considered that BDA is a reactive process, due to post-inflammatory or traumatic injury.[78] Subsequent studies have revealed that the majority of BDAs bear the BRAF V600E mutation, and some are associated with cholangiocarcinoma, which suggests the neoplastic process of BDA.[79,80] Similar to normal bile ducts, cytokeratin CK7 and CK19 are expressed in BDA, since these also express other foregut antigens, MUC6, MUC5AC and TTF2.[81] In order to distinguish BDA from iCCA, the immunohistochemistry for low Ki67 and wild-type p53 may be helpful. Some authors have reported to use EZH2 negativity and p16 positivity to assist in the BDA diagnosis.[82,83]

Biliary adenofibroma (BAF)

BAF is considered as a primary epithelial neoplasm with secondary stomal changes.[84] Although the morphology of BAF resembles the von Meyenburg complex, the immunohistochemical profile remains different. In addition to the expression of EMA, CK7, CK19 and CA19-9, BAF also presents with the amplifications of *CCND1* and *ERBB2*, suggesting its neoplastic nature. Furthermore, the *CDKN2A* mutation was reported in a case with malignant transformation.[85]

Non-neoplastic bile duct diseases

Primary biliary cholangitis (PBC)

The pathogenesis of PBC may be attributable to the genetic predisposition, environmental triggers, and complex interactions between the two.[86] One of the hallmarks of PBCs is serologically positive anti-mitochondrial autoantibodies (AMAs). However, AMA is not the only autoantibody detected in PBC. For example, disease-specific antinuclear antibodies (ANA) are present in approximately 33% of PBC patients, and present with the characteristic multiple nuclear dots (MND) or a rim-like/membranous (RLM) pattern in indirect immunofluorescence *in vitro*.[87] These patterns are diagnostic hallmarks of PBC, which can establish a diagnosis for PBC in patients without positive AMA (*e.g.* AMA-negative PBC patients with cholestasis).[88] The primary target antigens in RLM-pattern-associated antibodies are nuclear envelope proteins p62 and gp210. The presence of these antibodies is associated with a higher mortality rate, even in the patients without bilirubinemia at the time of diagnosis.[89,90]

Unlike various autoimmune liver diseases, potential autoreactive liver resident NK cells are enriched in the livers of PBC patients, and exhibit an increase in cytotoxic activities against autologous biliary epithelial cells.[91] Biliary epithelial cells express various antigens that allow interactions with the immune system, such as CD1d activates NK T cells.[92] Activated biliary epithelial cells are important for maintaining the characteristic inflammation of PBC *via* chemokine CCL19, cytokines, and vascular cell adhesion molecule-1.[93] However, to our knowledge, none of these markers have been proven for use as immunohistochemical markers for PBC diagnosis or prognosis.

Primary sclerosing cholangitis (PSC)

PSC is strongly associated with aberrant HLA alleles.[94] The strong link between PSC and inflammatory bowel disease leads to the "microbiota hypothesis." In the microbiota hypothesis, microbial molecules driven by intestinal dysbiosis reach the liver *via* portal circulation, and initiate a host of aberrant cholangiocytic behaviors (*e.g.* senescence).[95–97] The histopathologic hallmark of PSC is obliterative-concentric periductal loose fibrosis ("onion skinning"), while its radiological hallmark is the "beaded" biliary trees. During PSC development, cholangiocytic activation leads to the recruitment and infiltration of CD68+ macrophages. These macrophages produce proinflammatory cytokines that activate other immune system cells, and secrete profibrotic mediators, such as TGF-β and platelet-derived growth factor (PDGF), which lead to the activation of hepatic stellate cells.[98] Thus, cholangiocytes present with degenerative and atrophic changes, which in turn, causes biliary strictures, biliary occlusions ("bile duct scars"), and a "beaded" appearance on radiological imaging.[99] Periodic acid-Schiff staining with diastase can reveal the significantly thickened bile duct basement membrane, with a specificity of 95%, for PSC diagnosis.[100]

Molecular markers of other liver diseases

Liver fibrosis

Most chronic liver diseases can progress to liver fibrosis, and form fibrous scars. Hepatic stellate cells, which are activated by chronic liver injury, is the major source of fibrous scars in liver fibrosis.[101] Myofibroblasts, which are usually not present in normal livers, can be activated in the liver by chronic injury.[102] Hepatic fibrosis is the formation of fibrous scars, and is the result of excessive extracellular matrix proteins. The primary source of extracellular matrix proteins are myofibroblasts,[103] which are derived mainly from liver resident activated hepatic stellate cells and activated portal fibroblasts. Numerous molecular markers have been reported to be able to label myofibroblasts activated from hepatic stellate cells, including Desmin, CD146, CD105, GFAP, LRAT, Synemin, Synaptophysin, p75 (NGFR), PDGFRβ1, PPARγ, Adipor1, ADFP1, CD36, Cytoglobin, SPP1, LOX, LOXL2, NR1D2 and IL-17RA.[104,105] Myofibroblasts may also derive from activated portal fibroblasts.[106] The molecular markers that highlight myofibroblasts from this source are, as follows: THY1, Elastin, CD105, Cofilin, Fibulin2, Gremlin, NTPD2, Smoothelin, Calcitonin α, Mesothelin, uroplakin 1β, basonuclin 1, Asporin, Vitrin, IL-18R1 and COL15A1.[107–110] The modulation of TGF-β signaling *via* the TLR4-MyD88–NF-κB axis provides a link between proinflammatory and profibrogenic signals.[104] However, none of these molecular markers has been applied for diagnostic or therapeutic use.

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) and undifferentiated liver carcinoma (ULC)

The pathogenesis of cHCC-CCA and ULC remains unclear. cHCC-CCA is molecularly more similar to iCCA, when compared to HCC, and characteristic mutations have been identified in both HCC (*CTNNB1*) and iCCA (*KRAS* and *IDH1*).[111–113] cHCC-CCA with progenitor cell morphology is often positive for fetal-type growth factor SALL4.[75] Intermediate cell carcinoma of the liver is the term reserved for primary liver carcinoma with monotonous

morphological features. These are intermediated between hepatocellular and cholagniocytic cytologic features, and monotonous tumor cells express some HCC and iCCA markers.[114] Undifferentiated carcinoma lacks the definitive morphological and immunohistochemical features of any differentiation beyond the epithelial marker expression, and there is no evidence of specific carcinoma differentiation.[15]

Epithelioid hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) is a malignant endothelial neoplasm that comprises of epithelioid endothelial cells in myxohyaline or fibrous stroma. These tumor cells are positive for endothelial markers CD31, CD34, D2-40 and ERG.[115] Cytokeratin CK8 and CK18 may be patchy positive in tumor cells.[116] The characteristic feature of EHE is t(1;3)(p36;q25) translocation, leading to WWTR1-CAMTA1 gene fusion, and this has been identified in 90% of EHEs.[117–119] Immunostaining marker CAMTA1 has been used in studies, presenting a positive result in 85–90% of cases.[120,121]

Cautionary notes

The present review focused on the current and emerging molecular markers of liver diseases through the lens of pathogenesis. Various diseases, such as various types of cancers, share common signal pathways during its development (*e.g.* p53, c-Myc and APC) Thus, a number of markers may be found to be useful for diagnosing diseases of other organs. However, these alternations should be interpreted with caution, and combined with other clinicopathological data.

These current and emerging molecular markers remain largely untested in a large population, and warrant additional studies, particularly clinical trials, in order to determine the clinical values. Furthermore, as a limitation of the present study, a number of these markers were qualitative, and not quantitative, which may be subjected to interpretation bias.

Conclusions

The present review discussed the current and emerging molecular markers of common liver diseases. Specific focus was given on molecular, immunohistochemical and serological markers for diagnostic assistance and prognostic prediction, from a pathogenic perspective. Due to the rapid development of this field, it remains challenging to cover all markers for the diagnosis and prognosis of liver diseases. Nevertheless, markers in clinical practice and under investigation were reviewed in a wide spectrum of liver diseases (Table 1).[3,15–23,31–34,37–40,42,44,48,49,51,53,54,57,61,65,75,76,80,85,87,88,92,98,111,119,120] Machine learning tools and high-throughput proteomics would help reveal more-sensitive and more specific markers of liver diseases in the future.[7,8]

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Table 1. Molecular markers of common liver diseases

Disease	Marker	Test methods	Purpose	Status*	Reference
Hepatocellular carcinoma	CTNNB1 mutation	Sequencing	Diagnosis of early HCC	In practice	17
	TP53 mutation	Sequencing; Immunohistochemistry	Diagnosis	In practice	17
	HSP70	Immunohistochemistry	Diagnosis	In practice	18
	Glypican-3	Immunohistochemistry	Diagnosis	In practice	18
	Glutamine synthetase	Immunohistochemistry	Diagnosis	In practice	18
	Telomere length	Southern blotting	Diagnosis of dysplasia	Under Investigation	16
	DNAJB1-PRKACA translocation	FISH	Diagnosis of fibrolamellar variant	Under Investigation	3
	AFP	Serology	Screening	In practice (controversial)	19,20
	Lens culinaris-agglutinin-reactive fraction of AFP	Serology	Screening (with AFP)	Under investigation	21
	PIVKA-II	Serology	Screening (with AFP)	Under investigation	21
	des-gamma-carboxy prothrombin	Serology	Diagnosis of HCC	Under investigation	23
	Dickkopf-1	Serology	Diagnosis of HCC	Under investigation	22

(continued)

Table 1. *(continued)*

Disease	Marker	Test methods	Purpose	Status*	Reference
	CK19	immunohistochemistry	Prognosis of HCC	In practice	31–33
	miR-1180-3p	RT-PCR	Prognosis of HCC	Under investigation	34
	PD-L1	Immunohistochemistry	Therapy guidance	In practice	37
Hepatoblastoma	CTNNB1 exon 3 deletion	Sequencing	Diagnosis of HB	Under investigation	38
	AXIN1/2	PCR; Sequencing	Diagnosis of HB	Under investigation	39
	APC	Not specified	Diagnosis of HB	Under investigation	40
	Myc	Western blot; PCR	Diagnosis of HB	Under investigation	42
	β-Catenin	Immunohistochemistry	Diagnosis of HB	In practice	44
	Glutamine synthetase	Immunohistochemistry	Diagnosis of HB	In practice	44
	CK7/19	Immunohistochemistry	Diagnosis of HB	In practice	44
Focal nodular hyperplasia	Angioporitin	RT-PCR	Diagnosis of FNH	Under investigation	48
	serum amyloid A	Immunohistochemistry	Diagnosis of FNH	Under investigation	49
Hepatocellular adenoma	Liver fatty-acid binding protein	Immunohistochemistry	Diagnosis of HNF1A-inactivated HCA	In practice	15
	C-reactive protein/ serum amyloid A	Immunohistochemistry	Diagnosis of inflammatory HCA	In practice	15
	Glutamine synthetase	Immunohistochemistry	Diagnosis of β-catenin activated HCA	In practice	15
	TERT promoter mutation	Sequencing	malignant transformation of HCA	Under investigation	51
	PTGDS	Immunohistochemistry	sonic hedgehog HCA	Under Investigation	53
	Argininosuccinate synthase 1	Immunohistochemistry	ASS1-positive HCA	Under Investigation	15
Autoimmune hepatitis	Anti-smooth muscle antibodies	Serology	Type 1 AIH	In practice	54
	anti-liver-kidney microsomal-1/ anti-liver cytosol-1 antibody	Serology	Type 2 AIH	In practice	54
	MHC class II HLA DRB1*03/04	ELISA	Risk assessment of type 1 AIH	Under Investigation	57
	DRB1*07/03	ELISA	Risk assessment of type 2 AIH	Under Investigation	57
Metabolic-associated fatty liver disease (MAFLD)	PNPLA3	Western blotting	Risk assessment	Under Investigation	61
Hemochromatosis	HFE	Sequencing	Type 1	In practice	65
	HJV	Sequencing	Type 2a	In practice	65
	HAMP (hepcidin)	Sequencing	Type 2b	In practice	65
	TFR2	Sequencing	Type 3	In practice	65
	SLC40a1 (ferroportin)	Sequencing	Type 4a and 4b	In practice	65
Intrahepatic cholangiocarcinoma	CK7/19	Immunohistochemistry	Diagnosis	In practice	15

(continued)

Table 1. *(continued)*

Disease	Marker	Test methods	Purpose	Status*	Reference
	CD56	Immunohistochemistry	Small duct type	In practice	15
	C-reactive protein	Immunohistochemistry	Small duct type; Better prognosis	In practice	15,76
	MUC5AC/6	Immunohistochemistry	Large duct type	In practice	15
Bile duct adenoma	BRAF V600E mutation	PCR	Diagnosis	Under Investigation	80
Biliary adenomfibroma	CDKN2a	Sequencing	Malignant transformation	Under Investigation	85
Primary biliary cholangitis	Anti-mitochondrial autoantibodies	Serology	Diagnosis	In Practice	87
	anti-MND/RLM antibodies	Serology	Diagnosis	Under Investigation	88
	CD1d	Western blot	Diagnosis (label cholangiocytes)	Under Investigation	92
Primary Sclerosing cholangitis	CD68	Immunohistochemistry	Diagnosis (label macrophages)	Under Investigation	98
Liver fibrosis	TGFβ	RT-PCR	Diagnosis	Under Investigation	23
Combined hepatocellular-cholangiocarcinoma	CTNNB1	PCR	Diagnosis (for HCC component)	In practice	111
	KRAS/IDH1	PCR	Diagnosis (for iCCA component)	In practice	111
	SALL4	Immunohistochemistry	Diagnosis	Under Investigation	75
Epithelioid hemangioendothelioma	WWTR1-CAMTA1 gene fusion	RT-PCR	Diagnosis	In Practice	119
	CAMTA1	Immunohistochemistry	Diagnosis	In Practice	120

*Since there were no guidelines to distinguish whether the marker is used in practice or under investigation, the status of the marker was based on the experience of the authors. AFP, alpha-fetoprotein; APC, adenomatous polyposis coli; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CAMTA1, calmodulin-binding transcription activator 1; CD, cluster of differentiation; CDKN2a, cyclin-dependent kinase inhibitor 2A; CK, cytokeratin; CTNNB1, catenin beta 1; DNAJB1, DnaJ heat shock protein family (Hsp40) member B1; HFE, homeostatic iron regulator protein; HJV, hemojuvelin; HSP70, heat shock protein 70; IDH1, Isocitrate Dehydrogenase 1; KRAS, Kirsten rat sarcoma viral oncogene homolog; MHC, major histocompatibility complex; Myc, MYC Proto-Oncogene; PD-L1, programmed death-ligand 1; PIVKA-II, protein induced by vitamin K absence-II; PNPLA3, patatin-like phospholipase domain-containing protein 3; PRKACA , protein kinase CAMP-activated catalytic subunit alpha; PTGDS, prostaglandin D2 synthase; SALL4, spalt like transcription factor 4; TERT, telomerase reverse transcriptase; TFR2, transferrin receptor 2; TGFβ, transforming growth factor beta; TP53, tumor protein p53; WWTR, WW domain containing transcription regulator 1.

the writing of this work, or the decision to submit the same for publication.

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

YXL, GLG and LJZ contributed to the writing of the manuscript.

Reference

[1] Moon AM, Singal AG, Tapper EB. Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. Clin Gastroenterol Hepatol 2020; 18(12):2650–2666. doi:10.1016/j.cgh.2019.07.060, PMID:31401364.

[2] Wang HL, Kim CJ, Koo J, Zhou W, Choi EK, Arcega R, *et al*. Practical Immunohistochemistry in Neoplastic Pathology of the Gastrointestinal Tract, Liver, Biliary Tract, and Pancreas. Arch Pathol Lab Med 2017;141(9):1155–1180. doi:10.5858/arpa.2016-0489-RA, PMID:28854347.

[3] Graham RP, Yeh MM, Lam-Himlin D, Roberts LR, Terracciano L, Cruise MW, *et al*. Molecular testing for the clinical diagnosis of fibrolamellar carcinoma. Mod Pathol 2018;31(1):141–149. doi:10.1038/modpathol.2017.103, PMID:28862261.

[4] Zhang L. Biomarker discovery and validation in HCC diagnosis, prognosis, and therapy. In: Liu C (ed). Precision Molecular Pathology of Liver Cancer. Springer Nature; 2018.

[5] Yuan X, Duan SZ, Cao J, Gao N, Xu J, Zhang L. Noninvasive inflammatory markers for assessing liver fibrosis stage in autoimmune hepatitis patients. Eur J Gastroenterol Hepatol 2019;31(11):1467–1474. doi:10.1097/MEG.0000000000001437, PMID:31107735.

[6] Bioulac-Sage P, Cubel G, Balabaud C, Zucman-Rossi J. Revisiting the pathology of resected benign hepatocellular nodules using new immunohistochemical markers. Semin Liver Dis 2011;31(1):91–103. doi:10.1055/s-0031-1272837, PMID:21344354.

[7] Cui M, Cheng C, Zhang L. High-throughput proteomics: a methodological mini-review. Lab Invest 2022;3:1–12. doi:10.1038/s41374-022-00830-7, PMID:35922478.

[8] Cui M, Zhang DY. Artificial intelligence and computational pathology. Lab Invest 2021;101(4):412–422. doi:10.1038/s41374-020-00514-0, PMID:33454724.

[9] Global Burden of Disease Liver Cancer Collaboration. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol 2017;3(12):1683–1691. doi:10.1001/jamaoncol.2017.3055, PMID:28983565.

[10] El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132(7):2557–2576. doi:10.1053/j.gastro.2007.04.061, PMID:17570226.

[11] Bonilla Guerrero R, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. J Hepatol 2005;42(5):760–777. doi:10.1016/j.jhep.2005.02.005, PMID:15826727.

[12] Lan KH, Sheu ML, Hwang SJ, Yen SH, Chen SY, Wu JC, *et al*. HCV NS5A interacts with p53 and inhibits p53-mediated apoptosis. Oncogene 2002; 21(31):4801–4811. doi:10.1038/sj.onc.1205589, PMID:12101418.

[13] Jiang YF, He B, Li NP, Ma J, Gong GZ, Zhang M. The oncogenic role of NS5A of hepatitis C virus is mediated by up-regulation of survivin gene expression in the hepatocellular cell through p53 and NF-kappaB pathways. Cell Biol Int 2011;35(12):1225–1232. doi:10.1042/CBI20110102, PMID:21612579.

[14] Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 1991;350(6317):429–431. doi:10.1038/350429a0, PMID:1672732.

[15] WHO Classification of Tumours Editorial Board. Digestive System Tumours: WHO Classification of Tumours (Medicine) 5th Edition. World Health Organization; 2019.

[16] Lee YH, Oh BK, Yoo JE, Yoon SM, Choi J, Kim KS, *et al*. Chromosomal instability, telomere shortening, and inactivation of p21(WAF1/CIP1) in dysplastic nodules of hepatitis B virus-associated multistep hepatocarcinogenesis. Mod Pathol 2009;22(8):1121–1131. doi:10.1038/modpathol.2009.76, PMID:19465904.

[17] Marquardt JU, Seo D, Andersen JB, Gillen MC, Kim MS, Conner EA, *et al*. Sequential transcriptome analysis of human liver cancer indicates late stage acquisition of malignant traits. J Hepatol 2014;60(2):346–353. doi:10.1016/j.jhep.2013.10.014, PMID:24512821.

[18] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018; 69(1):182–236. doi:10.1016/j.jhep.2018.03.019, PMID:29628281.

[19] El-Nakeep S. Molecular and genetic markers in hepatocellular carcinoma: In silico analysis to clinical validation (current limitations and future promises). World J Gastrointest Pathophysiol 2022;13(1):1–14. doi:10.4291/wjgp.v13.i1.1, PMID:35116176.

[20] Sachar Y, Brahmania M, Dhanasekaran R, Congly SE. Screening for Hepatocellular Carcinoma in Patients with Hepatitis B. Viruses 2021;13(7):1318. doi:10.3390/v13071318, PMID:34372524.

[21] Kudo M. Management of Hepatocellular Carcinoma in Japan as a World-Leading Model. Liver Cancer 2018;7(2):134–147. doi:10.1159/000484619, PMID:29888204.

[22] Ryder SD. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. Gut 2003(Suppl 3):iii1–iii8. doi:10.1136/gut.52.suppl_3.iii1, PMID:12692148.

[23] Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, Saab S, *et al*. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 2008;23(10):1541–1548. doi:10.1111/j.1440-1746.2008.05395.x, PMID:18422961.

[24] Yang S, Yang L, Li X, Li B, Li Y, Zhang X, *et al*. New insights into autophagy in hepatocellular carcinoma: mechanisms and therapeutic strategies. Am J Cancer Res 2019;9(7):1329–1353. PMID:31392073.

[25] Liu L, Liao JZ, He XX, Li PY. The role of autophagy in hepatocellular carcinoma: friend or foe. Oncotarget 2017;8(34):57707–57722. doi:10.18632/oncotarget.17202, PMID:28915706.

[26] Ali MA, Matboli M, El-Khazragy N, Saber O, El-Nakeep S, Abdelzaher HM, *et al*. Investigating miRNA-661 and ATG4-B mRNA expression as potential biomarkers for hepatocellular carcinoma. Biomark Med 2018;12(3):245–256. doi:10.2217/bmm-2017-0273, PMID:29441798.

[27] Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. Cell 2017;169(7):1327–1341.e23. doi:10.1016/j.cell.2017.05.046, PMID:28622513.

[28] Liu J, Ma Z, Liu Y, Wu L, Hou Z, Li W. Screening of potential biomarkers in hepatitis C virus-induced hepatocellular carcinoma using bioinformatic analysis. Oncol Lett 2019;18(3):2500–2508. doi:10.3892/ol.2019.10578, PMID:31452738.

[29] Hou W, Qiao K, Huo Z, Du Y, Wang C, Syn WK. Association of IFNL3 rs12979860 polymorphism with HCV-related hepatocellular carcinoma susceptibility in a Chinese population. Clin Exp Gastroenterol 2019;12:433–439. doi:10.2147/CEG.S206194, PMID:31807049.

[30] Qin S, Wang J, Zhou C, Xu Y, Zhang Y, Wang X, *et al*. The influence of interleukin 28B polymorphisms on the risk of hepatocellular carcinoma among patients with HBV or HCV infection: An updated meta-analysis. Medicine (Baltimore) 2019;98(38):e17275. doi:10.1097/MD.0000000000017275, PMID:31568008.

[31] Kim H, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, *et al*. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. Hepatology 2011;54(5):1707–1717. doi:10.1002/hep.24559, PMID:22045674.

[32] Rhee H, Nahm JH, Kim H, Choi GH, Yoo JE, Lee HS, *et al*. Poor outcome of hepatocellular carcinoma with stemness marker under hypoxia: resistance to transarterial chemoembolization. Mod Pathol 2016;29(9):1038–1049. doi:10.1038/modpathol.2016.111, PMID:27312064.

[33] Tsuchiya K, Komuta M, Yasui Y, Tamaki N, Hosokawa T, Ueda K, *et al*. Expression of keratin 19 is related to high recurrence of hepatocellular carcinoma after radiofrequency ablation. Oncology 2011;80(3-4):278–288. doi:10.1159/000328448, PMID:21734420.

[34] Zhou Z, Zhou X, Jiang Y, Qiu M, Liang X, Lin Q, *et al*. Clinical significance

of miR-1180-3p in hepatocellular carcinoma: a study based on bioinformatics analysis and RT-qPCR validation. Sci Rep 2020;10(1):11573. doi:10.1038/s41598-020-68450-z, PMID:32665670.
[35] Yang K, Li J, Sun Z, Zhao L, Bai C. Retreatment with immune checkpoint inhibitors in solid tumors: a systematic review. Ther Adv Med Oncol 2020;12:1758835920975353. doi:10.1177/1758835920975353, PMID:33294036.
[36] Saung MT, Pelosof L, Casak S, Donoghue M, Lemery S, Yuan M, *et al*. FDA Approval Summary: Nivolumab Plus Ipilimumab for the Treatment of Patients with Hepatocellular Carcinoma Previously Treated with Sorafenib. Oncologist 2021;26(9):797–806. doi:10.1002/onco.13819, PMID:33973307.
[37] Xu H, Liang XL, Liu XG, Chen NP. The landscape of PD-L1 expression and somatic mutations in hepatocellular carcinoma. J Gastrointest Oncol 2021;12(3):1132–1140. doi:10.21037/jgo-21-251, PMID:34295562.
[38] Curia MC, Zuckermann M, De Lellis L, Catalano T, Lattanzio R, Aceto G, *et al*. Sporadic childhood hepatoblastomas show activation of beta-catenin, mismatch repair defects and p53 mutations. Mod Pathol 2008;21(1):7–14. doi:10.1038/modpathol.3800977, PMID:17962810.
[39] Taniguchi K, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, *et al*. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. Oncogene 2002;21(31):4863–4871. doi:10.1038/sj.onc.1205591, PMID:12101426.
[40] Yang A, Sisson R, Gupta A, Tiao G, Geller JI. Germline APC mutations in hepatoblastoma. Pediatr Blood Cancer 2018;65(4):e26892. doi:10.1002/pbc.26892, PMID:29251405.
[41] Czauderna P, Lopez-Terrada D, Hiyama E, Haberle B, Malogolowkin MH, Meyers RL. Hepatoblastoma state of the art: pathology, genetics, risk stratification, and chemotherapy. Curr Opin Pediatr 2014;26(1):19–28. doi:10.1097/MOP.0000000000000046, PMID:24322718.
[42] Cairo S, Armengol C, De Reynies A, Wei Y, Thomas E, Renard CA, *et al*. Hepatic stem-like phenotype and interplay of Wnt/beta-catenin and Myc signaling in aggressive childhood liver cancer. Cancer Cell 2008;14(6):471–484. doi:10.1016/j.ccr.2008.11.002, PMID:19061838.
[43] Lopez-Terrada D, Alaggio R, de Davila MT, Czauderna P, Hiyama E, Katzenstein H, *et al*. Towards an international pediatric liver tumor consensus classification: proceedings of the Los Angeles COG liver tumors symposium. Mod Pathol 2014;27(3):472–491. doi:10.1038/modpathol.2013.80, PMID:24008558.
[44] Ranganathan S, Lopez-Terrada D, Alaggio R. Hepatoblastoma and Pediatric Hepatocellular Carcinoma: An Update. Pediatr Dev Pathol 2020;23(2):79–95. doi:10.1177/1093526619875228, PMID:31554479.
[45] Wanless IR, Mawdsley C, Adams R. On the pathogenesis of focal nodular hyperplasia of the liver. Hepatology 1985;5(6):1194–1200. doi:10.1002/hep.1840050622, PMID:4065824.
[46] Rebouissou S, Bioulac-Sage P, Zucman-Rossi J. Molecular pathogenesis of focal nodular hyperplasia and hepatocellular adenoma. J Hepatol 2008;48(1):163–170. doi:10.1016/j.jhep.2007.10.003, PMID:17997499.
[47] Rebouissou S, Couchy G, Libbrecht L, Balabaud C, Imbeaud S, Auffray C, *et al*. The beta-catenin pathway is activated in focal nodular hyperplasia but not in cirrhotic FNH-like nodules. J Hepatol 2008;49(1):61–71. doi:10.1016/j.jhep.2008.03.013, PMID:18466996.
[48] Bioulac-Sage P, Rebouissou S, Sa Cunha A, Jeannot E, Lepreux S, Blanc JF, *et al*. Clinical, morphologic, and molecular features defining so-called telangiectatic focal nodular hyperplasias of the liver. Gastroenterology 2005;128(5):1211–1218. doi:10.1053/j.gastro.2005.02.004, PMID:15887105.
[49] Joseph NM, Ferrell LD, Jain D, Torbenson MS, Wu TT, Yeh MM, *et al*. Diagnostic utility and limitations of glutamine synthetase and serum amyloid-associated protein immunohistochemistry in the distinction of focal nodular hyperplasia and inflammatory hepatocellular adenoma. Mod Pathol 2014;27(1):62–72. doi:10.1038/modpathol.2013.114, PMID:23807780.
[50] Margolskee E, Bao F, de Gonzalez AK, Moreira RK, Lagana S, Sireci AN, *et al*. Hepatocellular adenoma classification: a comparative evaluation of immunohistochemistry and targeted mutational analysis. Diagn Pathol 2016;11:27. doi:10.1186/s13000-016-0475-5, PMID:26961851.
[51] Pilati C, Letouze E, Nault JC, Imbeaud S, Boulai A, Calderaro J, *et al*. Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. Cancer Cell 2014;25(4):428–441. doi:10.1016/j.ccr.2014.03.005, PMID:24735922.
[52] Evason KJ, Grenert JP, Ferrell LD, Kakar S. Atypical hepatocellular adenoma-like neoplasms with beta-catenin activation show cytogenetic alterations similar to well-differentiated hepatocellular carcinomas. Hum Pathol 2013;44(5):750–758. doi:10.1016/j.humpath.2012.07.019, PMID:23084586.
[53] Nault JC, Couchy G, Balabaud C, Morcrette G, Caruso S, Blanc JF, *et al*. Molecular Classification of Hepatocellular Adenoma Associates with Risk Factors, Bleeding, and Malignant Transformation. Gastroenterology 2017;152(4):880–894.e6. doi:10.1053/j.gastro.2016.11.042, PMID:27939373.
[54] Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, *et al*. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48(1):169–176. doi:10.1002/hep.22322, PMID:18537184.
[55] Manns MP, Griffin KJ, Sullivan KF, Johnson EF. LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. J Clin Invest 1991;88(4):1370–1378. doi:10.1172/JCI115443, PMID:1717511.
[56] Lapierre P, Hajoui O, Homberg JC, Alvarez F. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology 1999;116(3):643–649. doi:10.1016/s0016-5085(99)70186-1, PMID:10029623.
[57] Terziroli Beretta-Piccoli B, Mieli-Vergani G, Vergani D. Autoimmmune hepatitis. Cell Mol Immunol 2022;19(2):158–176. doi:10.1038/s41423-021-00768-8, PMID:34580437.
[58] Drozdz K, Nabrdalik K, Hajzler W, Kwiendacz H, Gumprecht J, Lip GYH. Metabolic-Associated Fatty Liver Disease (MAFLD), Diabetes, and Cardiovascular Disease: Associations with Fructose Metabolism and Gut Microbiota. Nutrients 2021;14(1):103. doi:10.3390/nu14010103, PMID:35010976.
[59] Gallego-Duran R, Albillos A, Ampuero J, Arechederra M, Banares R, Blas-Garcia A, *et al*. Metabolic-associated fatty liver disease: From simple steatosis toward liver cirrhosis and potential complications. Proceedings of the *Third Translational Hepatology Meeting*, organized by the Spanish Association for the Study of the Liver (AEEH). Gastroenterol Hepatol 2022. doi:10.1016/j.gastrohep.2022.02.005, PMID:35248669.
[60] Jonas W, Schurmann A. Genetic and epigenetic factors determining NAFLD risk. Mol Metab 2021;50:101111. doi:10.1016/j.molmet.2020.101111, PMID:33160101.
[61] BasuRay S, Wang Y, Smagris E, Cohen JC, Hobbs HH. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proc Natl Acad Sci USA 2019;116(19):9521–9526. doi:10.1073/pnas.1901974116, PMID:31019090.
[62] Zhao Z, Meng J, Su R, Zhang J, Chen J, Ma X, *et al*. Epitranscriptomics in liver disease: Basic concepts and therapeutic potential. J Hepatol 2020;73(3):664–679. doi:10.1016/j.jhep.2020.04.009, PMID:32330603.
[63] Zhang L, Guo GL. Gut-specific inhibition of PPARalpha as a novel approach of NAFLD treatment. Hepatology 2022. doi:10.1002/hep.32592, PMID:35633082.
[64] Kowdley KV, Brown KE, Ahn J, Sundaram V. ACG Clinical Guideline: Hereditary Hemochromatosis. Am J Gastroenterol 2019;114(8):1202–1218. doi:10.14309/ajg.0000000000000315, PMID:31335359.
[65] Girelli D, Busti F, Brissot P, Cabantchik I, Muckenthaler MU, Porto G. Hemochromatosis classification: update and recommendations by the BIOIRON Society. Blood 2022;139(20):3018–3029. doi:10.1182/blood.2021011338, PMID:34601591.
[66] Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. Lancet 2016;388(10045):706–716. doi:10.1016/S0140-6736(15)01315-X, PMID:26975792.
[67] Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loreal O. Haemochromatosis. Nat Rev Dis Primers 2018;4:18016. doi:10.1038/nrdp.2018.16, PMID:29620054.
[68] Aishima S, Oda Y. Pathogenesis and classification of intrahepatic cholangiocarcinoma: different characters of perihilar large duct type versus peripheral small duct type. J Hepatobiliary Pancreat Sci 2015;22(2):94–100. doi:10.1002/jhbp.154, PMID:25181580.
[69] Nakanuma Y, Sudo Y. Biliary tumors with pancreatic counterparts. Semin Diagn Pathol 2017;34(2):167–175. doi:10.1053/j.semdp.2016.12.013, PMID:28109714.

[70] Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest 2012;122(11):3914–3918. doi:10.1172/JCI63065, PMID:23023701.
[71] Sia D, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. Gastroenterology 2017;152(4):745–761. doi:10.1053/j.gastro.2016.11.048, PMID:28043904.
[72] Komuta M, Spee B, Vander Borght S, De Vos R, Verslype C, Aerts R, *et al*. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 2008;47(5):1544–1556. doi:10.1002/hep.22238, PMID:18393293.
[73] Sempoux C, Fan C, Singh P, Obeidat K, Roayaie S, Schwartz M, *et al*. Cholangiolocellular carcinoma: an innocent-looking malignant liver tumor mimicking ductular reaction. Semin Liver Dis 2011;31(1):104–110. doi:10.1055/s-0031-1272838, PMID:21344355.
[74] Nakanuma Y, Sato Y, Ikeda H, Harada K, Kobayashi M, Sano K, *et al*. Intrahepatic cholangiocarcinoma with predominant "ductal plate malformation" pattern: a new subtype. Am J Surg Pathol 2012;36(11):1629–1635. doi:10.1097/PAS.0b013e31826e0249, PMID:23073321.
[75] Moeini A, Sia D, Zhang Z, Camprecios G, Stueck A, Dong H, *et al*. Mixed hepatocellular cholangiocarcinoma tumors: Cholangiolocellular carcinoma is a distinct molecular entity. J Hepatol 2017;66(5):952–961. doi:10.1016/j.jhep.2017.01.010, PMID:28126467.
[76] Yeh YC, Lei HJ, Chen MH, Ho HL, Chiu LY, Li CP, *et al*. C-Reactive Protein (CRP) is a Promising Diagnostic Immunohistochemical Marker for Intrahepatic Cholangiocarcinoma and is Associated with Better Prognosis. Am J Surg Pathol 2017;41(12):1630–1641. doi:10.1097/PAS.0000000000000957, PMID:28945626.
[77] Rhee H, Ko JE, Chung T, Jee BA, Kwon SM, Nahm JH, *et al*. Transcriptomic and histopathological analysis of cholangiolocellular differentiation trait in intrahepatic cholangiocarcinoma. Liver Int 2018;38(1):113–124. doi:10.1111/liv.13492, PMID:28608943.
[78] Bhathal PS, Hughes NR, Goodman ZD. The so-called bile duct adenoma is a peribiliary gland hamartoma. Am J Surg Pathol 1996;20(7):858–864. doi:10.1097/00000478-199607000-00009, PMID:8669534.
[79] Pujals A, Amaddeo G, Castain C, Bioulac-Sage P, Compagnon P, Zucman-Rossi J, *et al*. BRAF V600E mutations in bile duct adenomas. Hepatology 2015;61(1):403–405. doi:10.1002/hep.27133, PMID:24634053.
[80] Pujals A, Bioulac-Sage P, Castain C, Charpy C, Zafrani ES, Calderaro J. BRAF V600E mutational status in bile duct adenomas and hamartomas. Histopathology 2015;67(4):562–567. doi:10.1111/his.12674, PMID:25704541.
[81] Hughes NR, Goodman ZD, Bhathal PS. An immunohistochemical profile of the so-called bile duct adenoma: clues to pathogenesis. Am J Surg Pathol 2010;34(9):1312–1318. doi:10.1097/PAS.0b013e3181ead722, PMID:20679879.
[82] Tsokos CG, Krings G, Yilmaz F, Ferrell LD, Gill RM. Proliferative index facilitates distinction between benign biliary lesions and intrahepatic cholangiocarcinoma. Hum Pathol 2016;57:61–67. doi:10.1016/j.humpath.2016.06.019, PMID:27396933.
[83] Sasaki M, Matsubara T, Kakuda Y, Sato Y, Nakanuma Y. Immunostaining for polycomb group protein EZH2 and senescent marker p16INK4a may be useful to differentiate cholangiolocellular carcinoma from ductular reaction and bile duct adenoma. Am J Surg Pathol 2014;38(3):364–369. doi:10.1097/PAS.0000000000000125, PMID:24487593.
[84] Varnholt H, Vauthey JN, Dal Cin P, Marsh Rde W, Bhathal PS, Hughes NR, *et al*. Biliary adenofibroma: a rare neoplasm of bile duct origin with an indolent behavior. Am J Surg Pathol 2003;27(5):693–698. doi:10.1097/00000478-200305000-00014, PMID:12717255.
[85] Thompson SM, Zendejas-Mummert B, Hartgers ML, Venkatesh SK, Smyrk TC, Mahipal A, *et al*. Malignant transformation of biliary adenofibroma: a rare biliary cystic tumor. J Gastrointest Oncol 2016;7(6):E107–E112. doi:10.21037/jgo.2016.09.14, PMID:28078134.
[86] Sarcognato S, Sacchi D, Grillo F, Cazzagon N, Fabris L, Cadamuro M, *et al*. Autoimmune biliary diseases: primary biliary cholangitis and primary sclerosing cholangitis. Pathologica 2021;113(3):170–184. doi:10.32074/1591-951X-245, PMID:34294935.
[87] Missoum H, Alami M, Bachir F, Arji N, Bouyahya A, Rhajaoui M, *et al*. Prevalence of autoimmune diseases and clinical significance of autoantibody profile: Data from National Institute of Hygiene in Rabat, Morocco. Hum Immunol 2019;80(7):523–532. doi:10.1016/j.humimm.2019.02.012, PMID:30807792.
[88] Terziroli Beretta-Piccoli B, Mieli-Vergani G, Vergani D, Vierling JM, Adams D, Alpini G, *et al*. The challenges of primary biliary cholangitis: What is new and what needs to be done. J Autoimmun 2019;105:102328. doi:10.1016/j.jaut.2019.102328, PMID:31548157.
[89] Yang F, Yang Y, Wang Q, Wang Z, Miao Q, Xiao X, *et al*. The risk predictive values of UK-PBC and GLOBE scoring system in Chinese patients with primary biliary cholangitis: the additional effect of anti-gp210. Aliment Pharmacol Ther 2017;45(5):733–743. doi:10.1111/apt.13927, PMID:28083929.
[90] Terziroli Beretta-Piccoli B, Mieli-Vergani G, Vergani D. The clinical usage and definition of autoantibodies in immune-mediated liver disease: A comprehensive overview. J Autoimmun 2018;95:144–158. doi:10.1016/j.jaut.2018.10.004, PMID:30366656.
[91] Shimoda S, Harada K, Niiro H, Shirabe K, Taketomi A, Maehara Y, *et al*. Interaction between Toll-like receptors and natural killer cells in the destruction of bile ducts in primary biliary cirrhosis. Hepatology 2011;53(4):1270–1281. doi:10.1002/hep.24194, PMID:21400555.
[92] Schrumpf E, Tan C, Karlsen TH, Sponheim J, Bjorkstrom NK, Sundnes O, *et al*. The biliary epithelium presents antigens to and activates natural killer T cells. Hepatology 2015;62(4):1249–1259. doi:10.1002/hep.27840, PMID:25855031.
[93] Barone F, Gardner DH, Nayar S, Steinthal N, Buckley CD, Luther SA. Stromal Fibroblasts in Tertiary Lymphoid Structures: A Novel Target in Chronic Inflammation. Front Immunol 2016;7:477. doi:10.3389/fimmu.2016.00477, PMID:27877173.
[94] Lazaridis KN, LaRusso NF. Primary Sclerosing Cholangitis. N Engl J Med 2016;375(12):1161–1170. doi:10.1056/NEJMra1506330, PMID:27653566.
[95] Yan C, Koda S, Wu J, Zhang BB, Yu Q, Netea MG, *et al*. Roles of Trained Immunity in the Pathogenesis of Cholangiopathies: A Therapeutic Target. Hepatology 2020;72(5):1838–1850. doi:10.1002/hep.31395, PMID:32463941.
[96] Dean G, Hanauer S, Levitsky J. The Role of the Intestine in the Pathogenesis of Primary Sclerosing Cholangitis: Evidence and Therapeutic Implications. Hepatology 2020;72(3):1127–1138. doi:10.1002/hep.31311, PMID:32394535.
[97] Shah A, Macdonald GA, Morrison M, Holtmann G. Targeting the Gut Microbiome as a Treatment for Primary Sclerosing Cholangitis: A Conceptional Framework. Am J Gastroenterol 2020;115(6):814–822. doi:10.14309/ajg.0000000000000604, PMID:32250997.
[98] Guicciardi ME, Trussoni CE, Krishnan A, Bronk SF, Lorenzo Pisarello MJ, O'Hara SP, *et al*. Macrophages contribute to the pathogenesis of sclerosing cholangitis in mice. J Hepatol 2018;69(3):676–686. doi:10.1016/j.jhep.2018.05.018, PMID:29802947.
[99] Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet 2013;382(9904):1587–1599. doi:10.1016/S0140-6736(13)60096-3, PMID:23810223.
[100] Colling R, Verrill C, Fryer E, Kartsonaki C, Wang LM, Chapman R, *et al*. Bile duct basement membrane thickening in primary sclerosing cholangitis. Histopathology 2016;68(6):819–824. doi:10.1111/his.12857, PMID:26333410.
[101] Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol 2021;18(3):151–166. doi:10.1038/s41575-020-00372-7, PMID:33128017.
[102] Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008;233(2):109–122. doi:10.3181/0707-MR-190, PMID:18222966.
[103] Friedman SL. Liver fibrosis — from bench to bedside. J Hepatol 2003;38(Suppl 1):S38–53. doi:10.1016/s0168-8278(02)00429-4, PMID:12591185.
[104] Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, *et al*. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13(11):1324–1332. doi:10.1038/nm1663, PMID:17952090.
[105] Friedman SL. Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88(1):125–172. doi:10.1152/physrev.00013.2007, PMID:18195085.
[106] Lemoinne S, Cadoret A, El Mourabit H, Thabut D, Housset C. Origins and functions of liver myofibroblasts. Biochim Biophys Acta 2013;1832(7):948–954. doi:10.1016/j.bbadis.2013.02.019, PMID:234

70555.
[107] Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, *et al*. Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci USA 2014;111(32):E3297–3305. doi:10.1073/pnas.1400062111, PMID:25074909.
[108] Dranoff JA, Wells RG. Portal fibroblasts: Underappreciated mediators of biliary fibrosis. Hepatology 2010;51(4):1438–1444. doi:10.1002/hep.23405, PMID:20209607.
[109] Koyama Y, Wang P, Liang S, Iwaisako K, Liu X, Xu J, *et al*. Mesothelin/mucin 16 signaling in activated portal fibroblasts regulates cholestatic liver fibrosis. J Clin Invest 2017;127(4):1254–1270. doi:10.1172/JCI88845, PMID:28287406.
[110] Bosselut N, Housset C, Marcelo P, Rey C, Burmester T, Vinh J, *et al*. Distinct proteomic features of two fibrogenic liver cell populations: hepatic stellate cells and portal myofibroblasts. Proteomics 2010;10(5):1017–1028. doi:10.1002/pmic.200900257, PMID:20049859.
[111] Sasaki M, Sato Y, Nakanuma Y. Mutational landscape of combined hepatocellular carcinoma and cholangiocarcinoma, and its clinicopathological significance. Histopathology 2017;70(3):423–434. doi:10.1111/his.13084, PMID:27634656.
[112] Liu ZH, Lian BF, Dong QZ, Sun H, Wei JW, Sheng YY, *et al*. Whole-exome mutational and transcriptional landscapes of combined hepatocellular cholangiocarcinoma and intrahepatic cholangiocarcinoma reveal molecular diversity. Biochim Biophys Acta Mol Basis Dis 2018;1864(6 Pt B):2360–2368. doi:10.1016/j.bbadis.2018.01.027, PMID:29408647.
[113] Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, *et al*. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. Nat Commun 2015;6:6120. doi:10.1038/ncomms7120, PMID:25636086.
[114] Brunt E, Aishima S, Clavien PA, Fowler K, Goodman Z, Gores G, *et al*. cHCC-CCA: Consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentation. Hepatology 2018;68(1):113–126. doi:10.1002/hep.29789, PMID:29360137.
[115] Miettinen M, Wang ZF, Paetau A, Tan SH, Dobi A, Srivastava S, *et al*. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. Am J Surg Pathol 2011;35(3):432–441. doi:10.1097/PAS.0b013e318206b67b, PMID:21317715.
[116] Miettinen M, Fetsch JF. Distribution of keratins in normal endothelial cells and a spectrum of vascular tumors: implications in tumor diagnosis. Hum Pathol 2000;31(9):1062–1067. doi:10.1053/hupa.2000.9843, PMID:11014572.
[117] Mendlick MR, Nelson M, Pickering D, Johansson SL, Seemayer TA, Neff JR, *et al*. Translocation t(1;3)(p36.3;q25) is a nonrandom aberration in epithelioid hemangioendothelioma. Am J Surg Pathol 2001;25(5):684–687. doi:10.1097/00000478-200105000-00019, PMID:11342784.
[118] Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, *et al*. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. Genes Chromosomes Cancer 2011;50(8):644–653. doi:10.1002/gcc.20886, PMID:21584898.
[119] Patel NR, Salim AA, Sayeed H, Sarabia SF, Hollingsworth F, Warren M, *et al*. Molecular characterization of epithelioid haemangioendotheliomas identifies novel WWTR1-CAMTA1 fusion variants. Histopathology 2015;67(5):699–708. doi:10.1111/his.12697, PMID:25817592.
[120] Doyle LA, Fletcher CD, Hornick JL. Nuclear Expression of CAMTA1 Distinguishes Epithelioid Hemangioendothelioma From Histologic Mimics. Am J Surg Pathol 2016;40(1):94–102. doi:10.1097/PAS.0000000000000511, PMID:26414223.
[121] Shibuya R, Matsuyama A, Shiba E, Harada H, Yabuki K, Hisaoka M. CAMTA1 is a useful immunohistochemical marker for diagnosing epithelioid haemangioendothelioma. Histopathology 2015;67(6):827–835. doi:10.1111/his.12713, PMID:25879300.