Journal of Clinical and Translational Hepatology **2024** vol. 12(1) | 52–61
DOI: 10.14218/JCTH.2023.00242

Original Article

Hepatocellular Ballooning is Due to Highly Pronounced Glycogenosis Potentially Associated with Steatosis and Metabolic Reprogramming

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Received: 18 May 2023 | **Revised:** 22 August 2023 | **Accepted:** 5 September 2023 | **Published online:** 19 October 2023

Abstract

Background and Aims: Hepatocellular ballooning is a common finding in chronic liver disease, mainly characterized by rarefied cytoplasm that often contains Mallory-Denk bodies (MDB). Ballooning has mostly been attributed to degeneration but its striking resemblance to glycogenotic/steatotic changes characterizing preneoplastic hepatocellular lesions in animal models and chronic human liver diseases prompts the question whether ballooned hepatocytes (BH) are damaged cells on the path to death or rather viable cells, possibly involved in neoplastic development. ***Methods:*** Using specimens from 96 cirrhotic human livers, BH characteristics were assessed for their glycogen/lipid stores, enzyme activities, and proto-oncogenic signaling cascades by enzyme- and immunohistochemical approaches with serial paraffin and cryostat sections. ***Results:*** BH were present in 43.8% of cirrhotic livers. Particularly pronounced excess glycogen storage of (glycogenosis) and/or lipids (steatosis) were characteristic, ground glass features and MDB were often observed. Decreased glucose-6-phosphatase, increased glucose-6-phosphate dehydrogenase activity and altered immunoreactivity of enzymes involved in glycolysis, lipid metabolism, and cholesterol biosynthesis were discovered. Furthermore, components of the insulin signaling cascade were upregulated along with insulin dependent glucose transporter glucose transporter 4 and the v-akt murine thymoma viral oncogene homolog/mammalian target of rapamycin signaling pathway associated with *de novo* lipogenesis. ***Conclusions:*** BH are hallmarked by particularly pronounced glycogenosis with facultative steatosis, many of their features being reminiscent of metabolic aberrations documented in preneoplastic hepatocellular lesions in experimental animals and chronic human liver diseases. Hence, BH are not damaged entities facing death but rather viable cells featuring metabolic reprogramming, indicative of a preneoplastic nature.

Citation of this article: Ribback S, Peters K, Yasser M, Prey J, Wilhelmi P, Su Q, *et al*. Hepatocellular Ballooning is Due to Highly Pronounced Glycogenosis Potentially Associated with Steatosis and Metabolic Reprogramming. J Clin Transl Hepatol 2024;12(1):52–61. doi: 10.14218/JCTH.2023.00242.

Introduction

Hepatocellular ballooning is a common but poorly understood alteration observed with a variety of acute and chronic conditions, such as alcoholic hepatitis, nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH), viral hepatitis, autoimmune hepatitis, chronic cholestasis, and toxic liver injury.[1–5] Ballooned hepatocytes (BH) are characterized by a rarefied edematous cytoplasm and a distinct diagnostic feature of NAFLD and NASH,[5] which are chronic liver diseases associated with the metabolic syndrome and the risk of progression to fibrosis, cirrhosis and hepatocellular carcinoma (HCC),[6,7] including clear-cell and steatohepatitic HCC.[8,9] NAFLD-associated HCC may appear against a noncirrhotic background.[10–13] Host metabolic status is also a major determinant of progression to HCC in cases of chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections.[14,15]

Most authors have interpreted hepatocellular ballooning as a sign of degeneration, increased fluid in the cytosol[16] causing lytic,[17] apoptotic[18] or oncotic cell death.[19] Accumulation of fat droplets may be a feature[5] with phospholipid-rich shells containing oxidized phosphatidylcholine and alterations of so-called "PAT" proteins regulating insulin-sensitive droplet lipase activity.[20,21] In NASH, lipotoxic effects have been suggested to elicit ballooning and apoptosis of hepatocytes.[16] BH

Keywords: Chronic liver disease; Carbohydrate metabolism; Insulin signaling; Preneoplasia; Metabolic reprogramming.

Abbreviations: Akt, v-akt murine thymoma viral oncogene homolog; BH, ballooned hepatocytes; CK, cytokeratin; DAB, diaminobenzidine; ERK, extracellular related kinase; FASN, fatty acid synthase; GGH, ground glass hepatocytes; GLUT4, glucose transporter 4; GSD, glycogenosis storing disease; G6Pase, glucose-6-phosphatase; G6PDH, glucose-6-phosphate dehydrogenase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; H&E, hematoxylin and eosin; HK, hexokinase; HMGCoAR, 3-hydroxy-3-methylglutaryl-CoA-reductase; IGLK, iso-glukokinase; IRS1, insulin receptor substrate 1; MDB, Mallory-Denk bodies; mTOR, mammalian target of rapamycin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PAS, periodic acid Schiff reaction; PBC, primary biliary cirrhosis; PKM2, pyruvate kinase 2; p-mTOR, phosphorylated-mammalian target of rapamycin; PSC, primary sclerosing cholangitis; qPCR, quantitative real-time polymerase chain reaction; RER, rough endoplasmic reticulum; SCD1, stearoyl-CoA desaturase; SER, smooth endoplasmic reticulum.

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Table 1. Underlying diseases in the 96 cirrhotic liver cases

Liver cirrhosis	*n/N* (%)
Alcoholic	35/96 (37)
Posthepatitic: HBV	15/96 (16)
Posthepatitic: HCV	27/96 (28)
Posthepatitic: autoimmune	3/96 (3)
Biliary: PBC, PSC	7/96 (7)
Cryptogenic	7/96 (7)
Other	4/96 (4)

of NAFLD patients were found to be strongly immunoreactive for sonic hedgehog, the level of expression correlating with the severity of liver damage, including hepatocellular ballooning.[22] However, caspase 9, a pivotal enzyme for mitochondrial pathway of apoptosis, is diminished in BH.[23] Thus, BH may not be damaged cells on the path to cellular demise, but rather functional in nature. Many BH contain Mallory-Denk bodies (MDB),[2–5] which can be induced experimentally in rodents by carcinogens and have been discussed as possible indicators of preneoplasia.[24] Whereas cytokeratins (CKs) 8/18 are predominant components of MDB, the remaining cytoplasm of BH shows a loss of CK8/18[4] as previously observed in preneoplastic clear glycogenotic hepatocytes.[25]

The striking resemblance of hepatocellular ballooning to acquired glycogenotic/steatotic changes characterizing preneoplastic and highly differentiated neoplastic clear and acidophilic (eosinophilic) cell populations discovered in experimental chemical,[26] hepadnaviral[27,28] and hormonal[29,30] hepatocarcinogenesis have remained largely unconsidered, despite corresponding changes being well documented in humans.[8,30–37] This also holds true for the hypertrophy of the smooth endoplasmic reticulum (SER) in eosinophilic ground glass hepatocytes (GGH).[8,30–33] The complexity of BH feature recognition due to a lack of appropriate biomarkers has been emphasized in recent considerations on the application of artificial intelligence-based imaging for diagnosis of NAFLD.[38,39]

We previously proposed that human BH may be particularly enlarged glycogenotic clear, acidophilic hepatocytes corresponding to those of preneoplastic cell populations in animal models.[8] We have tested that hypothesis using cytomorphological and cytochemical approaches. We realize that the cirrhotic livers investigated did not include cases of NAFLD and NASH, but the phenotypic similarity of hepatocellular ballooning in various chronic liver diseases, particularly the consistent decrease in CK8/18, the frequent occurrence of MDB, and recent findings in patients suffering from NAFLD justify the inclusion of these diseases as detailed in the discussion.

Methods

Liver specimens

Liver tissues were provided by the tissue bank of the Nationales Centrum für Tumorerkrankungen (NCT, Heidelberg, Germany; Project No. 2233) in accordance with regulations and with approval of the ethics committee of the University of Heidelberg. Samples were obtained from 96 explanted livers of patients with liver cirrhosis due to various chronic liver diseases, including alcohol abuse, HBV or HCV infection, autoimmune hepatitis, primary biliary and/or sclerosing hepatitis, Morbus Byler or Wilson disease, and congenital liver fibrosis, as summarized in Table 1.

Tissue processing

Liver sampling was conducted within 45 minutes after explantation. Specimens were routinely fixed in Carnoy's solution and embedded in paraffin.[33] For Oil-red-O staining, enzyme histochemistry, and immunohistochemistry, slices of approximately 1.5 × 1.5 × 0.5 cm were immediately frozen in −120°C isopentane and stored at −80°C. Serial 2–3 µm serial sections were stained with hematoxylin and eosin (H&E), and used for the demonstration of glycogen by the periodic acid Schiff reaction (PAS) or histochemical staining with various antibodies. To avoid any elution of glycogen during the preparation of the paraffin sections for the PAS reaction, 70% ethanol was used instead of the conventional water bath. Serial 10 µm cryostat sections of representative cases were used for staining with H&E, Oil-red-O, the PAS-reaction, and the enzyme- or immunohistochemical assay of G6Pase by the lead method[40] and G6PDH by the nitro blue tetrazolium method.[41] Serial cryostat sections were also incubated with a battery of primary antibodies listed in Supplementary Table 1. They were aldolase A, CK18, fatty acid synthase (FASN), 3-hydroxy-3-methylglutaryl-CoA-reductase (HMGCoAR), glukokinase (IGLK), insulin receptor, insulin receptor substrate 1 (IRS1), v-akt murine thymoma viral oncogene homolog (Pan-Akt), extracellular related kinase (PanERK), pyruvate kinase 2 (PKM2), phosphorylated mammalian target of rapamycin (p-mTOR), Ras, raf1, and stearoyl-CoA desaturase (SCD1). Endogenous peroxidase was quenched with 1% hydrogen peroxide and positive reactivity was identified using an Ultravision LP detection system, alkaline phosphatase polymer, and fast red chromogen or horseradish peroxidase polymer and diaminobenzidine as chromogen substrates (Thermo Fisher Scientific, Waltham, MA, USA). Formalin-fixed, paraffin-embedded serial sections (5 µm thick) were immunostained for antigens with an automated system (Leica Biosystems, Wetzlar, Germany). Signal intensity in ballooned hepatocytes was estimated semiquantitatively by comparison with corresponding surrounding unaltered liver tissue. For negative controls, primary antibodies were omitted.

For western blotting, primary antibodies against p-Akt (S473), pan-Akt, PKM2, H-Ras, Erk1/2, IRS1, p-mTOR, and beta-actin were used (details are given in Supplementary Table 2). For quantitative real-time polymerase chain reaction (qRT-PCR), primers for human ERK1, ERK2, IRS1, KRAS, HRAS, FASN, PKM2, AKT1, AKT2 and ribonucleic acid ribosomal 18S (RNR-18) genes were used as the housekeeping control. Details are given in Supplementary File 1.

Electron microscopy

Cryostat sections (50 µm thick) were fixed in 2.5% glutaraldehyde and cut into 2 mm^2 pieces with a razor blade and embedded in Glycid Ether 100. Sectioning with a Leica ultratome to 500 and 750 nm thick semithin sections was followed by staining as described by Richardson *et al*.[42] Ultrathin 70–90 nm sections were contrasted with uranyl acetate and lead citrate and examined with a Libra 120 electron microscope (Carl Zeiss, Jena, Germany).

Quantification of BH, GGH and MDB

Fractions (%) of BH and GGH, were evaluated in H&E sections from 37 representative cases with BH, and from 15 representative cases with GGH. The average volume fraction was semiquantitatively estimated from the fraction of all H&E-stained hepatocytes at ×100 magnification, using a NIKON DS-2MV digital camera and NIKON NIS Elements Imaging Software Package 4.0. Percentage (%) was calculated as the number of BH /total numbers of hepatocytes

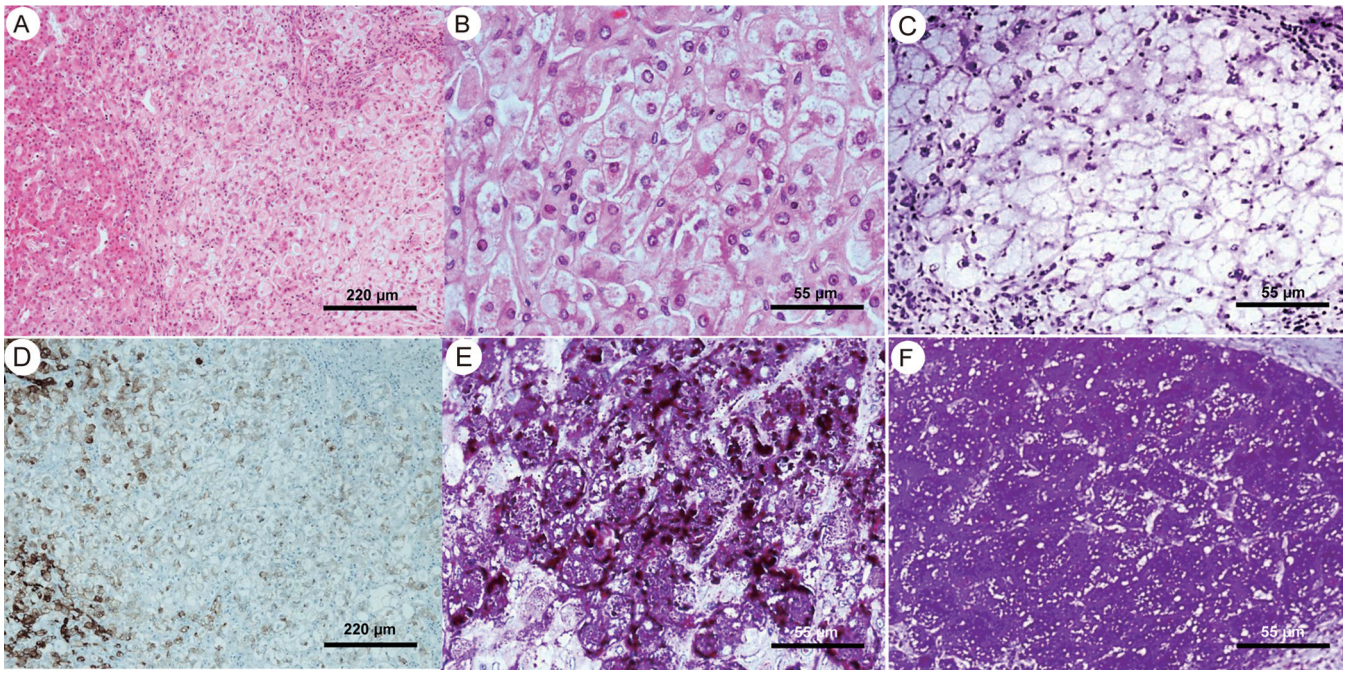

Fig. 1. Serial paraffin sections of cirrhotic liver from a patient with alcoholic steatohepatitis. (A) Parenchymal portion showing ballooned (right part) in contrast to normal hepatocytes (left part) (B) higher magnification showing many ballooned hepatocytes with clear or slightly eosinophilic (ground glass) cytoplasm and prominent nuclei (H&E), corresponding (D) to loss of expression of CK18; and (E) excessive storage of glycogen (PAS). Serial paraffin sections of cirrhotic liver from an HBV case are shown. Group of ballooned hepatocytes with typical clear cytoplasm, highly condensed nuclear chromatin (C, H&E) and massive glycogen content (F, PAS). Labeled scale bars are included in every picture.

×100 and number of GGH/total number of hepatocytes ×100. MDB occurrence was reported as 1 for present and 0 for not present.

Results

Frequency, morphology and ultrastructure of BH, MDB, and GGH

BH and MDB: BH (Figs. 1, 2) were detected in 42 of the 96 explanted cirrhotic livers (44%). Of the positive cases, 30% were associated with alcoholic steatohepatitis, 24% with HBV, 16% with HCV and 14% with chronic cholangitis (Supplementary Table 3). BH were also detected in glycogenotic clear-cell and steatohepatitic HCC (Fig. 3A, B). BH may be associated with inflammatory infiltrates but frequently appeared without any spatial relationship to inflammatory cells.

The average volume fraction of BH in the liver parenchyma was 14 ± 2.4 vol. % (mean ± SEM). The cytoplasm of BH was mostly clear, or weakly eosinophilic (ground glass feature) after staining with H&E, and was strongly positive for glycogen, Characteristically, BH formed small foci or stood out as particularly pronounced glycogenotic cells within extended parenchymal areas composed of less altered glycogenotic clear and/or ground glass cells (Fig. 1). In relatively glycogen-poor cirrhotic nodules, BH were predominantly lined up near the fibrotic septae (Fig. 2). Electron micrographs showed a massive accumulation of glycogen alpha particles in the cytoplasmic matrix (Fig. 4A, B) in GGH, often located in the immediate vicinity of SER-membranes. The pronounced accumulation of the glycogen with and without SER-proliferation resulted in marked cellular enlargement and pushed the rough endoplasmic reticulum (RER) and associated mitochondria to peripheral and paranuclear regions. Glycogen accumulation was frequently accompanied by the appearance of lipid droplets (Fig. 4A). MDB were detected in 16 of 96 liver specimens (17%), most frequently in cases of alcoholic steatohepatitis (44%) and HCV (31%) and but in smaller amounts in cases of HBV (13%) and chronic cholangitis (13%) (Supplementary Table 3). MDB occurred in the cytoplasm of BH as amorphous hyaline structures, highlighted by ubiquitin staining (Fig. 2D, E) appearing as electron dense, tightly arranged fuzzy filaments under in electron micrographs (Fig. 4A, f). Figure 3A shows a particularly impressive case of glycogenotic clear-cell HCC characterized by extensive populations of large BH (Fig. 3A, a, c, d) storing excess glycogen (b) and occasionally also containing fat vacuoles and MDB (Fig. 3A, d). Transition from large glycogenotic BHs to smaller, slightly basophilic neoplastic cell populations poor in or free of glycogen, were evident (Fig. 3A, c, d).

GGH: GGH characterized by a homogeneous or reticular eosinophilic, more or less PAS-positive cytoplasm (Figs. 1B, D, and 2D, F) were present in 15 of 96 (16%), with the highest rates in cases of HBV (33%) or HCV (27%), alcoholic steatohepatitis (13%), and in one case each of primary biliary cholangitis (PBC) and Morbus Byler (Table 2) with an average volume fraction of 17.7 ± 4.6 vol. % (mean ± SEM). Intermediate forms between clear glycogenotic BH and GGH were evident in many places (Fig. 2D, F). Compared with clear glycogenotic BH, the glycogen content of GGH was usually less pronounced, but significant amounts of glycogen particles were evident (Fig. 4B, a) often near SER-membranes (Fig. 4B, b). Glycogen-rich hepatocytes containing "ergastoplasm pockets" were observed,[30] poor in, or completely free of, glycogen particles, but rich in ribosomes (Fig. 4B, c, d).

Enzyme histochemistry

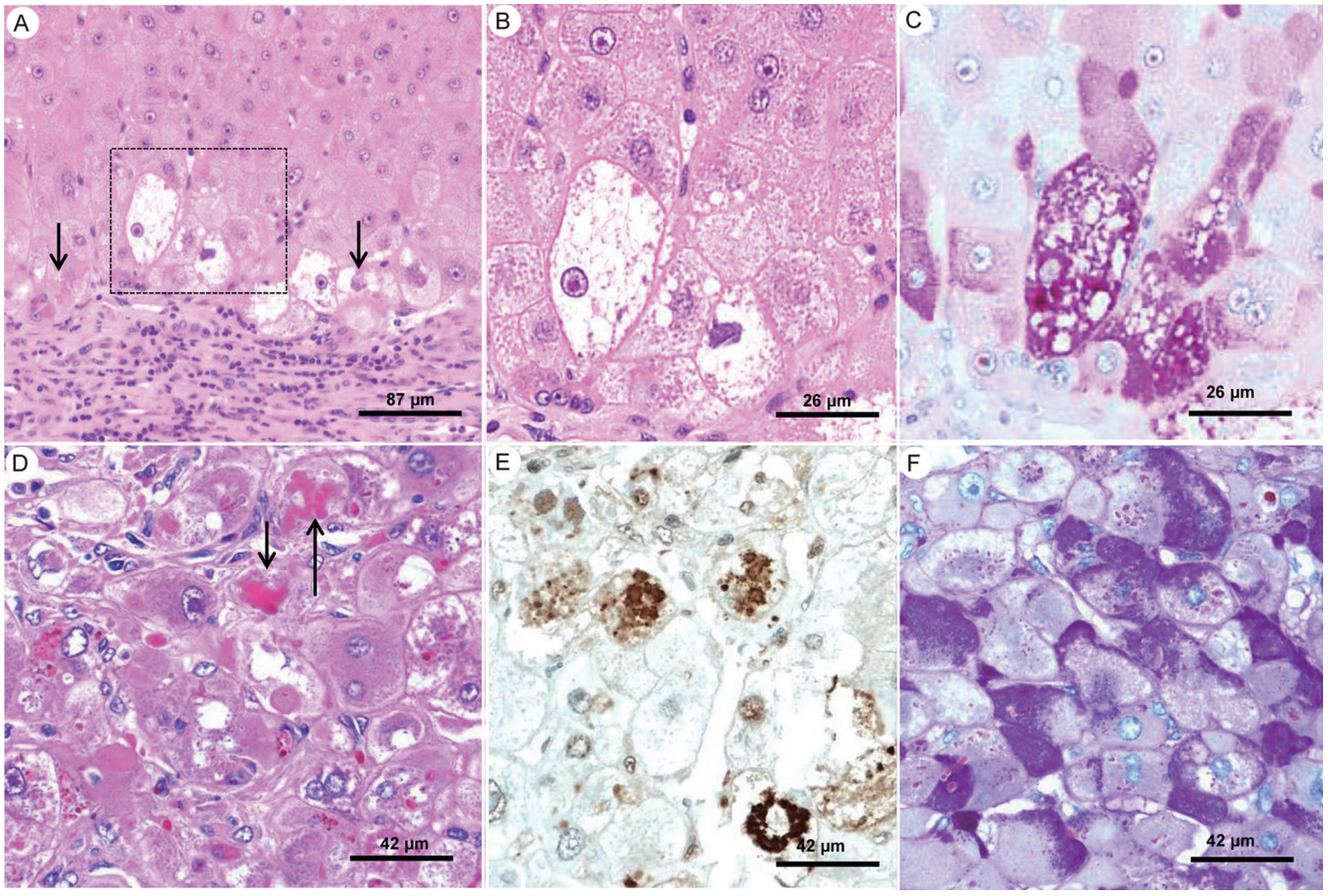

Fig. 2. Serial paraffin sections of cirrhotic liver from an HBV case. The portion of a cirrhotic nodule is shown containing several large ballooned hepatocytes with clear cytoplasm (A, B, H&E) excess glycogen storage (C, PAS) with fat vacuoles, sometimes also MDB in addition (A, H & E, arrows). Serial paraffin sections of cirrhotic liver from a patient with alcoholic steatohepatitis are shown. Several ballooned hepatocytes show clear cytoplasm and often also MDB (arrows) (D, H&E) positive for ubiquitin staining (E). In addition, eosinophilic GGH with variable glycogen content (F, PAS). Labeled scale bars are included in each picture.

G6Pase activity was decreased in glycogenotic BH compared with hepatocytes in the parenchyma of control livers. In contrast, G6PDH activity, the rate limiting enzyme of the pentose phosphate pathway, was strongly increased (Fig. 5).

Immunohistochemistry, western blot, and qPCR

Human clear-cell foci (CCF) of the liver, which have been described by our group,[34] store large glycogen particles and upregulate proto-oncogenic pathways like AKT/mTOR, Ras/raf and metabolic alterations, with upregulation of glycolysis and lipogenesis. Due to the morphologic similarity of hepatocytes in CCF to BHs we investigated analysis of these proteins also in BH: In serial cryosections, pronounced glycogen storage in BH was correlated with changes in carbohydrate and lipid metabolism, including increased immunoreactivity of the glycolytic enzymes glucokinase (IGLK), aldolase A and PK-M_2, enzymes of lipid biosynthesis FASN and SCD1, and cholesterol biosynthesis (HMGCoAR). In addition, several molecular changes related to insulin signaling pathways were observed, including enhanced immunoreactions compared with the surrounding liver parenchyma were noted for the insulin receptor, IRS1, Ras, raf-1, PanERK, the insulin dependent glucose transporter GLUT4, and AKT/mTOR (Fig. 6). Overexpression of pAKT, PKM2, H-Ras, Erk1/2 and IRS1 was also found by western blotting and quantification of cDNA revealed higher levels of PKM2, FASN, IRS1, HRAS and ERK1,2 in liver specimen with BH in comparison to control liver tissue without BH (Supplementary Figs. 1 and 2).

Discussion

In agreement with previous studies, considerably enlarged (ballooned) hepatocytes (BH) with clear, vacuolated or eosinophilic cytoplasm, pronounced loss of CK8/18, and frequently harboring CK8/18-containing MDB, were found to be common in cirrhotic livers of patients with various chronic liver diseases. After appropriate preservation during preparation, we were able to demonstrate that the typical appearance of BH was mainly due to particularly pronounced excessive storage of glycogen (glycogenosis), often associated with steatosis and ground glass features. According to biochemical studies, the capacity of hepatocytes to store glycogen is limited, and glucose that cannot be stored as glycogen is converted to fat by *de novo* lipogenesis.[43,44] In principle, the phenotype of BH corresponds to that of usually less enlarged glycogenotic/steatotic hepatocytes described as frequent components of preneoplastic focal and premalignant nodular liver lesions developing in various chronic liver diseases.[32]

The morphological, enzyme- and immunohistochemical characteristics of BH all point to similarities with focal preneoplastic liver lesions in animal models of chemical, hormonal, and viral hepatocarcinogenesis, characterized by con-

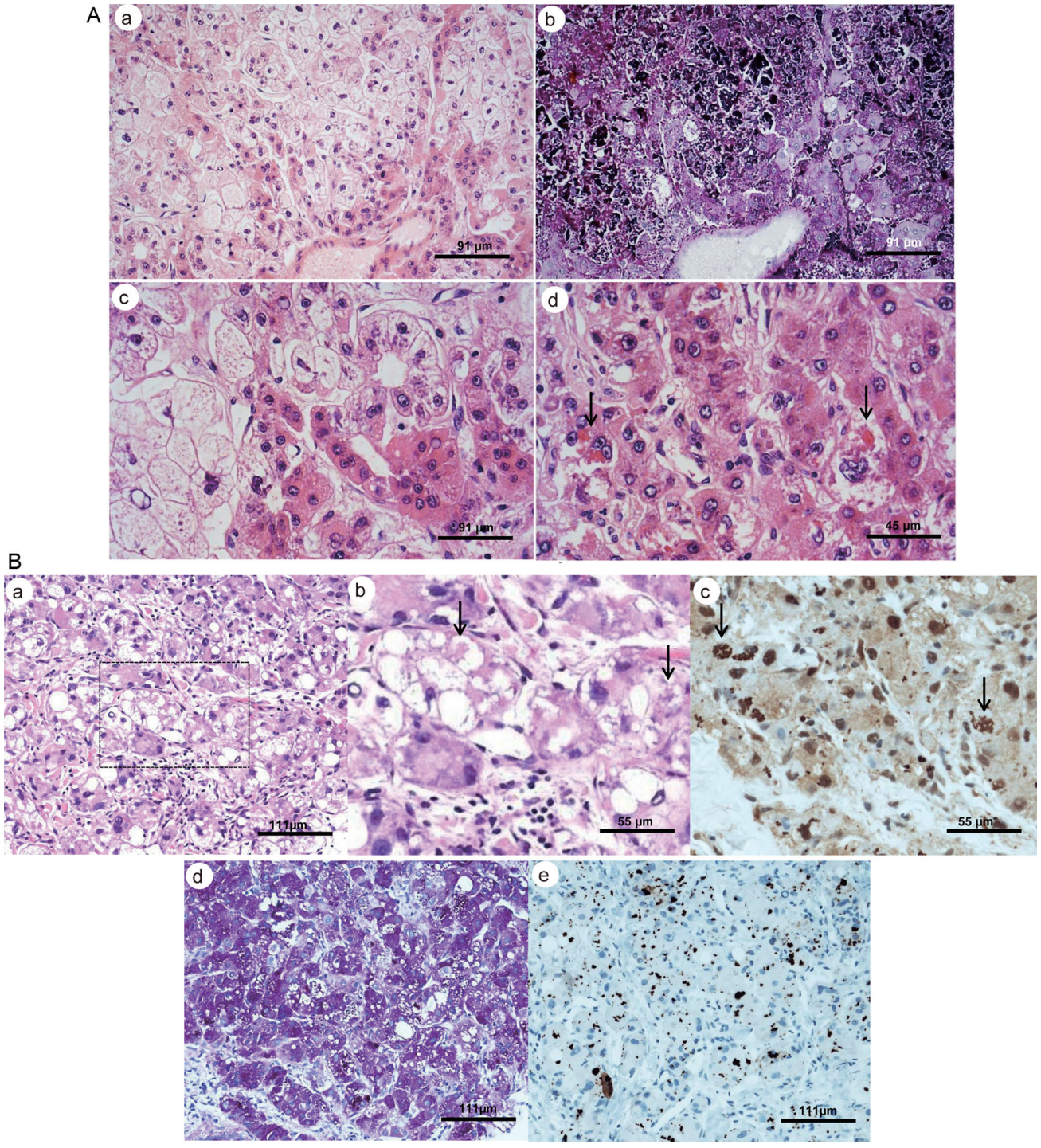

Fig. 3. Findings in hepatocellular carcinomas with gylcogenos and steatosis. A: Serial paraffin sections of glycogenotic clear-cell HCC from an HCV case. Tumor portions containing many ballooned clear cells (a, H&E) excessively storing glycogen (b, PAS), and showing gradual transitions from clear glycogenotic to glycogen poor, slightly basophilic tumor cells (c, H&E) occasionally containing MDB (arrows) (d, H&E). B: Serial paraffin sections of hepatocellular carcinoma with steatohepatitic features. Ballooning of clear neoplastic hepatocytes (a and square with higher magnification in b, H&E), occasionally containing MDB as demonstrated by ubiquitin staining (c), and showing excessive storage of glycogen, some fat vacuoles (d, PAS), and loss of cytokeratin 18 expression (e). Labeled scale bars are included in each picture.

sistent activation of insulin dependent glycogenotic[45–48] and lipogenic pathways,[48,49] and an early elevation of glucose-6-phosphate (G6P), a central metabolite channeling glucose into the different pathways of carbohydrate metabolism.[45] In line with these findings, cultured human hepatocytes and hepatoma cells lacking CK8/18 have an elevated glucose uptake, G6P formation and glycogen production compared with their counterparts containing CK8/18. These effects are fur-

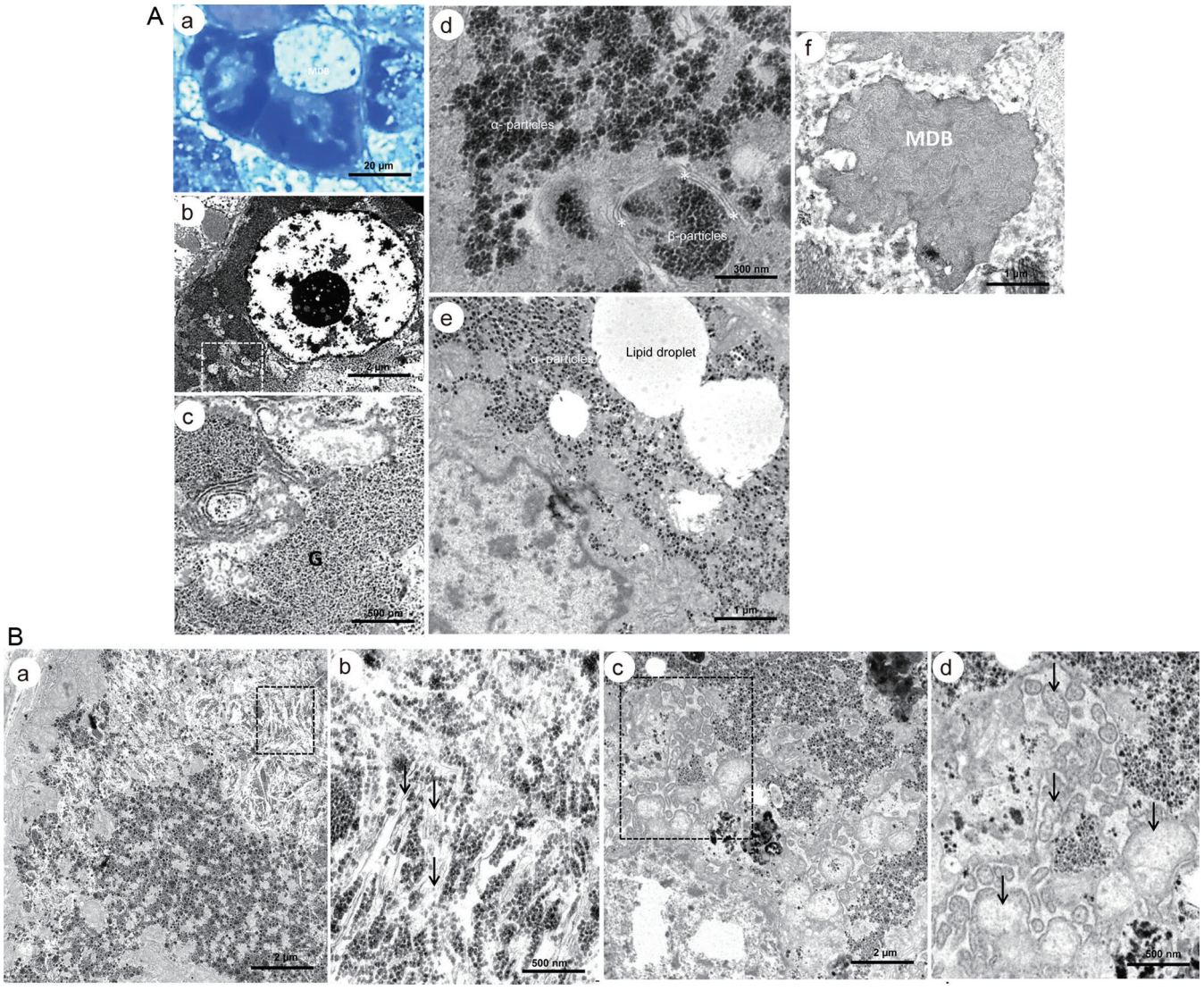

Fig. 4. Serial semithin resin sections of cirrhotic liver from patients with HBV observed by microscopy. Ultrastructural findings. (A) Ballooned hepatocytes with strong PAS-positive cytoplasm (a), corresponding to glycogen particles (G) in the cytoplasm (b), magnified segment square in (c). (d) Accumulation of glycogen as alpha particles in the cytoplasmic matrix and as beta particles in glycogenosome (*). (e) Combination of glycogen accumulation and lipid droplets. (f) MDB with tightly arranged fuzzy filaments. (B) Portions of GGH showing extended SER (a and square with higher magnification in b, arrows) alternating with layers of alpha or beta glycogen particles. Glycogen-rich hepatocytes with formation of unusual RER complexes (ergastoplasma pockets) which are poor in, or completely free of, glycogen particles but rich in ribosomes. c, and with higher magnification in d, arrows). Labeled scale bars are included in each picture. MDB, Mallory-Denk bodies.

ther increased by activation of the insulin signaling cascade through the mTOR-dependent pathway, along with an augmentation in cell proliferation.[50]

The combination of glycogenosis and SER-hypertrophy in BH corresponds to observations in preneoplastic or highly differentiated neoplastic cell populations in animal models,[30,26–28] human inborn glycogenosis type 1 (GSD I)[51] and focal and nodular lesions in chronic human liver diseases.[24] Evidence for a metabolically active G6P pool in the lumen of liver microsomal vesicles and a reversible G6P transporter in liver microsomal membranes has been provided.[52,53] In GSD I, microsomal vesicles contain relatively high intravesicular G6P levels,[52] suggesting that accumulation of G6P and SER-hypertrophy are closely related. The pronounced accumulation of glycogen, with and without SER-proliferation, in BH pushes the remaining organelles to peripheral and paranuclear parts of the cytoplasm, leading to a dislocation and relative reduction of the RER and mitochondria, possibly contributing to the miscommunication which has been proposed as an early and causal trigger of hepatic insulin resistance and steatosis.[54] High frequency and long persistence of ballooning hepatocytes has actually been shown to be associated with glucose intolerance in patients with severe obesity.[55]

Acquired hepatocellular glycogenosis in animal models of hepatocarcinogenesis and chronic human liver diseases finds a counterpart in different types of GSD, such as GSD I due to G6Pase deficiency and GSD VI triggered by a genetic defect of glycogen phosphorylase, both conveying a high risk of hepatocellular adenomas and carcinomas.[8,30,51] GSDI has been modeled in mice by targeted deletion of the G6Pase gene eliciting glycogenosis, steatosis, and eventually hepatocellular neoplasms.[56]

Table 2. Frequency of BH, MDB and GGH in 96 cirrhotic liver samples

Cirrhotic etiology	BH, *n/N* (%)	MDB, *n/N* (%)	GGH, *n/N* (%)
Liver cirrhosis	42/96 (44)	16/96 (17)	15/96 (16)
Alcoholic	11/42 (30)	7/16 (44)	2/15 (13)
Posthepatitic: HBV	6/42 (16)	2/16 (13)	5/15 (33)
Posthepatitic: HCV	9/42 (24)	5/16 (31)	4/15 (27)
Posthepatitic: autoimmune	1/42 (3)	0/16 (0)	0/15 (0)
Biliary: PBC, PSC	5/42 (14)	2/16 (13)	1/15 (7)
Cryptogenic	3/42 (8)	1/16 (6)	3/15 (20)
Others	2/42 (5)	0/16 (0)	1/15 (7)

For acquired murine glycogenosis, it has been shown that the early decrease of G6Pase and glycogen phosphorylase activities is maintained during progression to hepatocellular neoplasms. Ever increasing activities of G6PDH and glycolytic enzymes indicate a fundamental metabolic shift toward the pentose phosphate pathway and the Warburg-type glycolysis, providing energy and precursors for nucleic acid synthesis and cell proliferation.[45] Cytomorphological correlates of this metabolic reprogramming are gradual reduction of the glycogen initially stored in excess, frequent transient steatosis, and increase in ribosomes (basophilia) typical for the advanced neoplastic phenotype.[30] In mice exposed to diethylnitrosamine or subjected to a liver-specific knockout of G6Pase, the accumulated glycogen has been shown to undergo liquid-liquid phase separation and formation of glycogen-liquid droplets, which via inhibition of Hippo signaling stimulates cell proliferation and promotes hepatocarcinogenesis.[57] Elimination of glycogen accumulation abrogated tumor development, whereas increasing glycogen storage accelerated tumorigenesis.

In BH, glycogenosis is associated with high expression of glycolytic enzymes, corresponding to the glycogen shunt, which couples glycogen synthesis and breakdown pathways to the Warburg effect.[58] During the progression from preneoplastic glycogenotic to glycogen-poor neoplastic cell populations, this process is linked to an isoenzyme shift from the pyruvate kinase characteristic of normal hepatocytes (PK-M_1) to PK-M_2,[45,59] which is critical for regulating the glycogen shunt flux.[58] A regulatory mechanism of the glycogen shunt implicated in metabolic reprogramming has also been observed in Myc1 knockout mice and hepatic cell lines, showing progressive accumulation of glycogen and a redistribution of glucose from glycogen to other metabolic pathways, including the pentose phosphate pathway and glycolysis.[60] In addition to pyruvate kinase, hexokinase undergoes an isoenzyme shift during neoplastic pro-

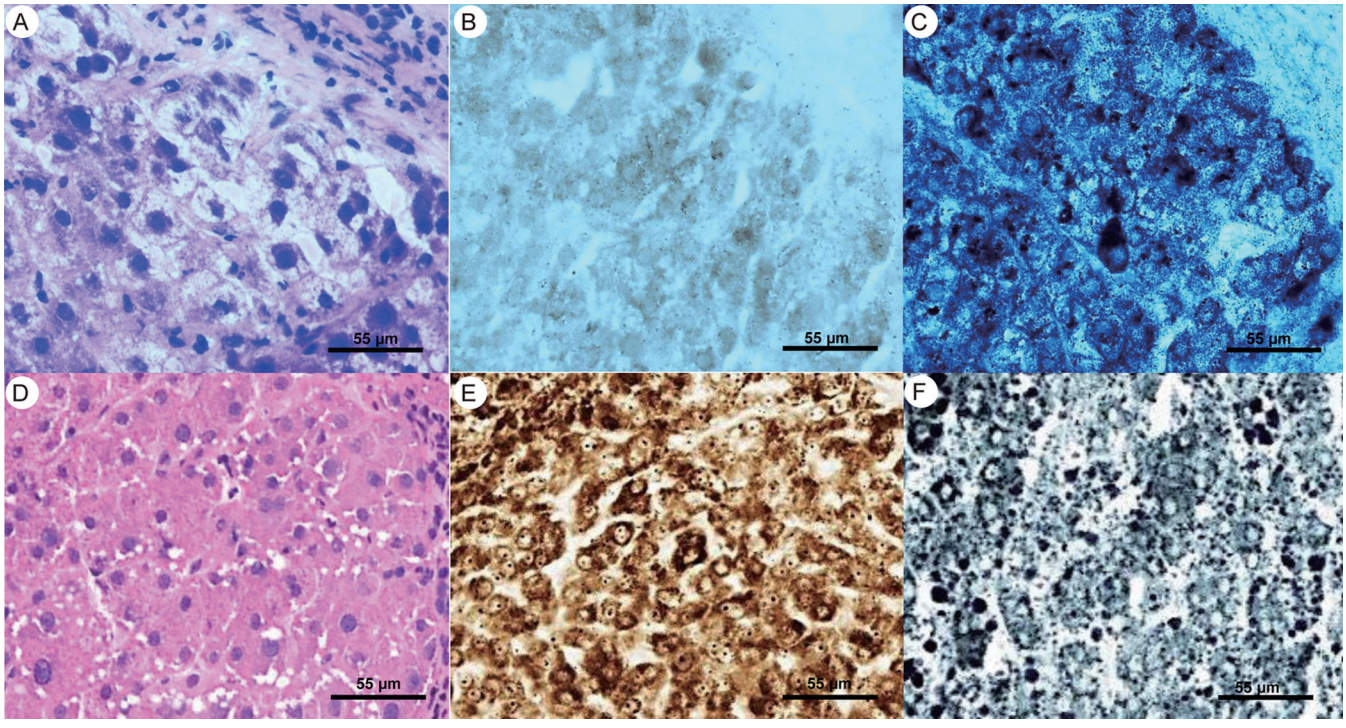

Fig. 5. Serial cryostat sections of cryptogenic cirrhotic liver and normal liver specimens comparing histological and enzyme histochemical properties. Cirrhotic liver nodule with ballooned hepatocytes (A, H&E) showing decreased activity of glucose-6-phosphatase (B) and increased activity of glucose-6-phosphate dehydrogenase (C) compared with normal liver parenchyma (D, H&E) (E and F). Labeled scale bars are included in each picture.

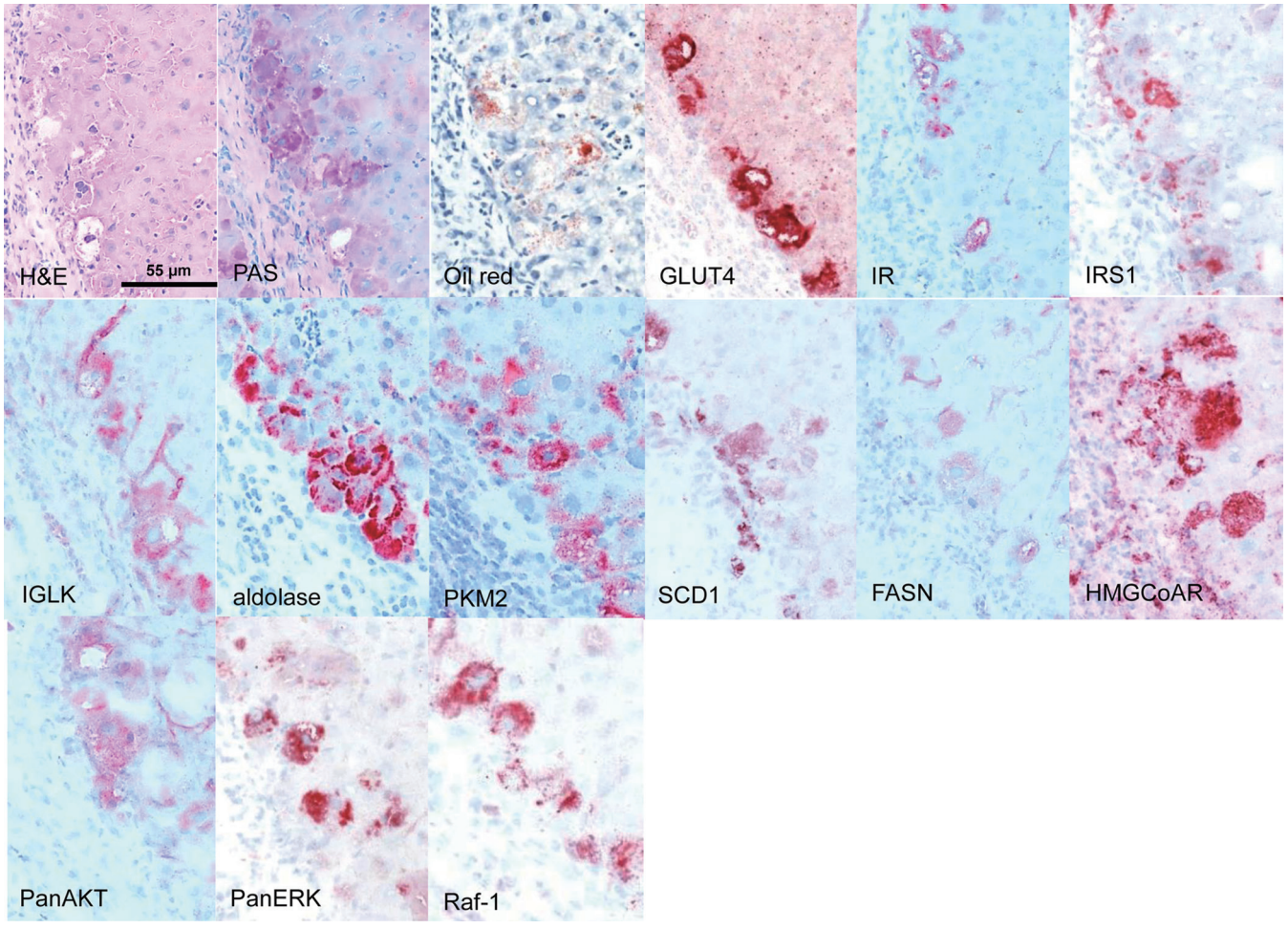

Fig. 6. Serial cryostat sections of part of a cirrhotic liver nodule from a HBV case. Several ballooned hepatocytes (H&E) with excess stored glycogen (PAS) and/or fat (Oil red) and showing overexpression of glucose transporter GLUT4, insulin receptor, IRS1, the glycolytic enzymes IGLK, aldolase A, PKM2, lipogenic enzymes like SCD1, FASN, HMGCoAR and upregulation of the proto-oncogenic pathways AKT/mTOR, ras/raf-1 and PanERK. The labeled scale bar included in H&E picture is representative of all images. H&E, hematoxylin and eosin; PAS, periodic acid Schiff reaction; GLUT4, glucose transporter 4; IRS1, insulin receptor substrate 1; IR, insulin receptor; IGLK, iso-glukokinase; PKM2, pyruvate kinase 2; SCD1, stearoyl-CoA desaturase; FASN, fatty acid synthase; HMGCoAR, 3-hydroxy-3-methylglutaryl-CoA-reductase; AKT, v-akt murine thymoma viral oncogene homolog; ERK, extracellular related kinase.

gression. The low-affinity hexokinase (glucokinase/HK IV), is replaced by the high affinity hexokinase (HKII) during neoplastic transformation of rat hepatocytes in vivo and *in vitro*.[45] Evidence for heightened HKII in human hepatocellular dysplasia and HCC also exists.[61]

In most animal models, glycogenotic/steatotic or purely steatotic hepatocytes appear only at later time points during progression from the initial hepatocellular glycogenosis to poorly differentiated HCC.[28,30] Similar observations were made in type 2 diabetes and GSD I, which are both at high risk of neoplastic development.[8,44] Thus, dysfunction of glucose metabolism may progress to NAFLD and eventually lead to hepatocellular neoplasms, suggesting that the combination of glycogenosis and steatosis, or steatosis alone, in BH are secondary rather than primary metabolic changes occurring predominantly during progression. Applying the PAS-reaction to paraffin sections of routinely processed liver biopsies from more than 2,000 children and adult patients, Allende et al.[62] found that a focal or diffuse glycogenosis of the liver parenchyma is common in NAFLD. They also observed BH with a particularly pronounced excessive storage of glycogen, but in contrast to our observations the large majority of BH did not contain visible glycogen. Statistical evaluation revealed that glycogenosis was independently associated with a high grade of ballooning, and the presence of MDB and decreased steatosis and fibrosis. Hence, the authors concluded that glycogenosis may have a protective effect on disease progression. We would infer from the findings in different animal models, human type 2 diabetes, and GSDI[8,44] that glycogenosis without steatosis and fibrosis indicates an earlier stage. The discrepancy in our observations and inference may be due to methodological differences in tissue preparation. Whereas Allende and colleagues used routinely processed liver biopsies collected from different laboratories, our specimens were not only fixed in Carnoýs solution preserving the glycogen, but were also cut and further processed without water contact to avoid elution.

Observations in both human and animal models agree that hepatocarcinogenesis may proceed with or without liver fibrosis and cirrhosis, depending on the severity of accompanying unspecific necroinflammatory changes elicited by the respective oncogenic agents.[31] The fact that HCC in chronic liver diseases, including NAFLD and NASH,[9–12] frequently ap-

pear without a background of fibrosis or cirrhosis implies that it is the metabolic dysfunction in altered hepatocytes, rather than the facultative necroinflammatory changes resulting in fibrosis and cirrhosis, which are essential for neoplastic development. The demonstration of pronounced glycogenotic and steatotic metabolic aberrations in BH comparable to those emerging early during hepatocarcinogenesis under a variety of conditions strongly suggests a preneoplastic or early neoplastic nature. Pronounced glycogenosis may serve as an appropriate biomarker for BH, particularly solving the dilemma recently emphasized by Li et al.[39] to distinguish BH from hepatocellular edema.

Acknowledgments

We thank Dr Doris Mayer and Dr Malcolm Moore for valuable suggestions and critical reading of the manuscript, and gratefully acknowledge technical assistance by Stefanie Bockhahn (electron microscopy), Cathrin Müller (immunohistochemistry), Katrin Sokolowski (serial paraffin sections).

Funding

This research was funded by the Deutsche Forschungsgemeinschaft (DFG), grant numbers RI 2695/1-1 and RI 2695/1-3. The funders played no role in the design of the study, the collection, analysis, and interpretation of the data and writing of the manuscript.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the manuscript, and interpretation of data (SR, PB), acquisition of data and performing of experiments (SR, KP, MY, JP, PW, QS, PB), and revising the manuscript for important intellectual content (SR, FD, PB). All authors have read and agreed to the published version of the manuscript.

Ethical statement

This study was carried out in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of the University of Heidelberg. Liver tissues were provided by the tissue bank of the Nationales Centrum für Tumorerkrankungen (NCT, Heidelberg, Germany; Project No. 2233). The individual consent for this retrospective analysis was waived.

Data sharing statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

References

[1] Review by an international group. Alcoholic liver disease: morphological manifestations. Lancet 1981;1(8222):707–11. PMID:6110925.
[2] French SW, Nash J, Shitabata P, Kachi K, Hara C, Chedid A, *et al*. Pathology of alcoholic liver disease. VA Cooperative Study Group 119. Semin Liver Dis 1993;13(2):154–169. doi:10.1055/s-2007-1007346, PMID:8393214.
[3] Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24(1):3–20. doi:10.1055/s-2004-823098, PMID:15085483.
[4] Lackner C, Gogg-Kamerer M, Zatloukal K, Stumptner C, Brunt EM, Denk H. Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. J Hepatol 2008;48(5):821–828. doi:10.1016/j.jhep.2008.01.026, PMID:18329127.
[5] Caldwell S, Ikura Y, Dias D, Isomoto K, Yabu A, Moskaluk C, *et al*. Hepatocellular ballooning in NASH. J Hepatol 2010;53(4):719–723. doi:10.1016/j.jhep.2010.04.031, PMID:20624660.
[6] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019;16(7):411–428. doi:10.1038/s41575-019-0145-7, PMID:31028350.
[7] Salomao M, Yu WM, Brown RS Jr, Emond JC, Lefkowitch JH. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. Am J Surg Pathol 2010;34(11):1630–1636. doi:10.1097/PAS.0b013e3181f31caa, PMID:20975341.
[8] Bannasch P, Ribback S, Su Q, Mayer D. Clear cell hepatocellular carcinoma: origin, metabolic traits and fate of glycogenotic clear and ground glass cells. Hepatobiliary Pancreat Dis Int 2017;16(6):570–594. doi:10.1016/S1499-3872(17)60071-7, PMID:29291777.
[9] Bralet MP, Régimbeau JM, Pineau P, Dubois S, Loas G, Degos F, *et al*. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. Hepatology 2000;32(2):200–204. doi:10.1053/jhep.2000.9033, PMID:10915724.
[10] Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, Vilgrain V, *et al*. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009;49(3):851–859. doi:10.1002/hep.22734, PMID:19115377.
[11] Ertle J, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, *et al*. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer 2011;128(10):2436–2443. doi:10.1002/ijc.25797, PMID:21128245.
[12] Yasui K, Hashimoto E, Komorizono Y, Koike K, Arii S, Imai Y, *et al*. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. Clin Gastroenterol Hepatol 2011;9(5):428–433. doi:10.1016/j.cgh.2011.01.023, PMID:21320639.
[13] Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, *et al*. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008;135(1):111–121. doi:10.1053/j.gastro.2008.03.073, PMID:18505690.
[14] Leslie J, Geh D, Elsharkawy AM, Mann DA, Vacca M. Metabolic dysfunction and cancer in HCV: Shared pathways and mutual interactions. J Hepatol 2022;77(1):219–236. doi:10.1016/j.jhep.2022.01.029, PMID:35157957.
[15] Yip WW, Burt AD. Alcoholic liver disease. Semin Diagn Pathol 2006;23(3-4):149–160. doi:10.1053/j.semdp.2006.11.002, PMID:17355088.
[16] Scheuer PJ, Lefkowitch JH. Liver Biopsy Interpretation. 7th ed. Elsevier-Saunders; 2005.
[17] Crawford JM. Histologic findings in alcoholic liver disease. Clin Liver Dis 2012;16(4):699–716. doi:10.1016/j.cld.2012.08.004, PMID:23101978.
[18] Ikura Y, Ohsawa M, Suekane T, Fukushima H, Itabe H, Jomura H, *et al*. Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease: impact on disease progression. Hepatology 2006;43(3):506–514. doi:10.1002/hep.21070, PMID:16496325.
[19] Fujii H, Ikura Y, Arimoto J, Sugioka K, Iezzoni JC, Park SH, *et al*. Expression of perilipin and adipophilin in nonalcoholic fatty liver disease; relevance to oxidative injury and hepatocyte ballooning. J Atheroscler Thromb 2009;16(6):893–901. doi:10.5551/jat.2055, PMID:20032580.
[20] Straub BK, Stoeffel P, Heid H, Zimbelmann R, Schirmacher P. Differential pattern of lipid droplet-associated proteins and de novo perilipin expression in hepatocyte steatogenesis. Hepatology 2008;47(6):1936–1946. doi:10.1002/hep.22268, PMID:18393390.
[21] Guy CD, Suzuki A, Zdanowicz M, Abdelmalek MF, Burchette J, Unalp A, *et al*. Hedgehog pathway activation parallels histologic severity of injury and fibrosis in human nonalcoholic fatty liver disease. Hepatology 2012;55(6):1711–1721. doi:10.1002/hep.25559, PMID:22213086.
[22] Kakisaka K, Cazanave SC, Werneburg NW, Razumilava N, Mertens JC, Bronk SF, *et al*. A hedgehog survival pathway in 'undead' lipotoxic hepatocytes. J Hepatol 2012;57(4):844–851. doi:10.1016/j.jhep.2012.05.011, PMID:22641094.
[23] Nakanuma Y, Ohta G. Is mallory body formation a preneoplastic change? A study of 181 cases of liver bearing hepatocellular carcinoma and 82 cases of cirrhosis. Cancer 1985;55(10):2400–2404. doi:10.1002/1097-0142(19850515)55:10<2400::aid-cncr2820551017>3.0.co;2-b, PMID:2985233.
[24] Su Q, Zerban H, Otto G, Bannasch P. Cytokeratin expression is reduced in glycogenotic clear hepatocytes but increased in ground-glass cells in chronic human and woodchuck hepadnaviral infection. Hepatology 1998;28(2):347–359. doi:10.1002/hep.510280209, PMID:9695996.
[25] Bannasch P, Haertel T, Su Q. Significance of hepatic preneoplasia in risk identification and early detection of neoplasia. Toxicol Pathol 2003;31(1):134–139. doi:10.1080/01926230390173923, PMID:12597458.
[26] Toshkov I, Chisari FV, Bannasch P. Hepatic preneoplasia in hepatitis B virus transgenic mice. Hepatology 1994;20(5):1162–72. PMID:7927248.
[27] Radaeva S, Li Y, Hacker HJ, Burger V, Kopp-Schneider A, Bannasch P. Hepadnaviral hepatocarcinogenesis: in situ visualization of viral antigens, cytoplasmic compartmentation, enzymic patterns, and cellular proliferation in preneoplastic hepatocellular lineages in woodchucks. J Hepatol 2000;33(4):580–600. doi:10.1034/j.1600-0641.2000.033004580.x, PMID:11059863.
[28] Dombrowski F, Bannasch P, Pfeifer U. Hepatocellular neoplasms induced by low-number pancreatic islet transplants in streptozotocin diabetic rats. Am J Pathol 1997;150(3):1071–87. PMID:9060843.

[29] Nuernberger V, Mortoga S, Metzendorf C, Burkert C, Ehricke K, *et al*. Hormonally Induced Hepatocellular Carcinoma in Diabetic Wild Type and Carbohydrate Responsive Element Binding Protein Knockout Mice. Cells 2021;10(10):2787. doi:10.3390/cells10102787, PMID:34685767.
[30] Bannasch P, Mayer D, Hacker HJ. Hepatocellular glycogenosis and hepatocarcinogenesis. Biochim Biophys Acta 1980;605(2):217–245. doi:10.1016/0304-419x(80)90005-0, PMID:6994813.
[31] Bannasch P. Pathogenesis of hepatocellular carcinoma: sequential cellular, molecular, and metabolic changes. Prog Liver Dis 1996;14:161–197. PMID:9055578.
[32] Su Q, Benner A, Hofmann WJ, Otto G, Pichlmayr R, Bannasch P. Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia. Virchows Arch 1997;431(6):391–406. doi:10.1007/s004280050116, PMID:9428927.
[33] Callea F, Giovannoni I, Stefanelli M, Villanacci V, Lorini G, Francalanci P. Glycogenotic hepatocellular carcinoma with glycogen-ground-glass hepatocytes: histological, histochemical and microbiochemical characterization of the novel variant. Histopathology 2012;60(6):1010–1012. doi:10.1111/j.1365-2559.2011.04168.x, PMID:22321151.
[34] Ribback S, Calvisi DF, Cigliano A, Sailer V, Peters M, Rausch J, *et al*. Molecular and metabolic changes in human liver clear cell foci resemble the alterations occurring in rat hepatocarcinogenesis. J Hepatol 2013;58(6):1147–1156. doi:10.1016/j.jhep.2013.01.013, PMID:23348238.
[35] Lefkowitch JH, Lagana SM, Kato T. Hepatocellular Carcinoma in Noncirrhotic Liver with Glycogenotic Foci: Basic Science Meets Genomic Medicine. Semin Liver Dis 2015;35(4):450–456. doi:10.1055/s-0035-1568986, PMID:26676821.
[36] Cano L, Cerapio JP, Ruiz E, Marchio A, Turlin B, Casavilca S, *et al*. Liver clear cell foci and viral infection are associated with non-cirrhotic, non-fibrolamellar hepatocellular carcinoma in young patients from South America. Sci Rep 2018;8(1):9945. doi:10.1038/s41598-018-28286-0, PMID:30061721.
[37] Glushko T, Kushchayev SV, Trifanov D, Salei A, Morales D, Berry G, *et al*. Focal Hepatic Glycogenosis in a Patient With Uncontrolled Diabetes Mellitus Type 1. J Comput Assist Tomogr 2018;42(2):230–235. doi:10.1097/RCT.0000000000000673, PMID:28937487.
[38] Brunt EM, Clouston AD, Goodman Z, Guy C, Kleiner DE, Lackner C, *et al*. Complexity of ballooned hepatocyte feature recognition: Defining a training atlas for artificial intelligence-based imaging in NAFLD. J Hepatol 2022;76(5):1030–1041. doi:10.1016/j.jhep.2022.01.011, PMID:35090960.
[39] Li YY, Zheng TL, Xiao SY, Wang P, Yang WJ, Jiang LL, *et al*. Hepatocytic ballooning in non-alcoholic steatohepatitis: Dilemmas and future directions. Liver Int 2023;43(6):1170–1182. doi:10.1111/liv.15571, PMID:37017559.
[40] Wachstein M, Meisel E. On the histochemica demonstration of glucose-6-phosphatase. J Histochem Cytochem 1956;4(6):592. doi:10.1177/4.6.592.
[41] Rudolph G, Klein HJ. [Histochemical demonstration and distribution of glucose-6-phosphate dehydrogenase in normal rat organs]. Histochemie 1964;4(3):238–251. doi:10.1007/BF00290868, PMID:5889987.
[42] Richardson KC, Jarrett L, Finke EH. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol 1960;35:313–323. doi:10.3109/10520296009114754, PMID:13741297.
[43] McDevitt RM, Bott SJ, Harding M, Coward WA, Bluck LJ, Prentice AM. De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women. Am J Clin Nutr 2001;74(6):737–746. doi:10.1093/ajcn/74.6.737, PMID:11722954.
[44] Rajas F, Gautier-Stein A, Mithieux G. Glucose-6 Phosphate, A Central Hub for Liver Carbohydrate Metabolism. Metabolites 2019;9(12):282. doi:10.3390/metabo9120282, PMID:31756997.
[45] Bannasch P, Klimek F, Mayer D. Early bioenergetic changes in hepatocarcinogenesis: preneoplastic phenotypes mimic responses to insulin and thyroid hormone. J Bioenerg Biomembr 1997;29(4):303–313. doi:10.1023/a:1022438528634, PMID:9387091.
[46] Evert M, Sun J, Pichler S, Slavova N, Schneider-Stock R, Dombrowski F. Insulin receptor, insulin receptor substrate-1, Raf-1, and Mek-1 during hormonal hepatocarcinogenesis by intrahepatic pancreatic islet transplantation in diabetic rats. Cancer Res 2004;64(21):8093–8100. doi:10.1158/0008-5472.CAN-04-2040, PMID:15520221.
[47] Aleem E, Nehrbass D, Klimek F, Mayer D, Bannasch P. Upregulation of the insulin receptor and type I insulin-like growth factor receptor are early events in hepatocarcinogenesis. Toxicol Pathol 2011;39(3):524–543. doi:10.1177/0192623310396905, PMID:21411721.
[48] Evert M, Calvisi DF, Evert K, De Murtas V, Gasparetti G, Mattu S, *et al*. V-AKT murine thymoma viral oncogene homolog/mammalian target of rapamycin activation induces a module of metabolic changes contributing to growth in insulin-induced hepatocarcinogenesis. Hepatology 2012;55(5):1473–1484. doi:10.1002/hep.25600, PMID:22271091.
[49] Calvisi DF, Wang C, Ho C, Ladu S, Lee SA, Mattu S, *et al*. Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. Gastroenterology 2011;140(3):1071–1083. doi:10.1053/j.gastro.2010.12.006, PMID:21147110.
[50] Mathew J, Loranger A, Gilbert S, Faure R, Marceau N. Keratin 8/18 regulation of glucose metabolism in normal versus cancerous hepatic cells through differential modulation of hexokinase status and insulin signaling. Exp Cell Res 2013;319(4):474–486. doi:10.1016/j.yexcr.2012.11.011, PMID:23164509.
[51] Spycher MA, Gitzelmann R. Glycogenesis type I (glucose 6-phosphatase deficiency): ultrastructural alterations of hepatocytes in a tumor bearing liver. Virchows Arch B Cell Pathol 1971;8:133–142. doi:10.1007/BF02893521.
[52] Bánhegyi G, Marcolongo P, Fulceri R, Hinds C, Burchell A, Benedetti A. Demonstration of a metabolically active glucose-6-phosphate pool in the lumen of liver microsomal vesicles. J Biol Chem 1997;272(21):13584–13590. doi:10.1074/jbc.272.21.13584, PMID:9153206.
[53] Gerin I, Van Schaftingen E. Evidence for glucose-6-phosphate transport in rat liver microsomes. FEBS Lett 2002;517(1-3):257–260. doi:10.1016/s0014-5793(02)02640-6, PMID:12062448.
[54] Beaulant A, Dia M, Pillot B, Chauvin MA, Ji-Cao J, Durand C, *et al*. Endoplasmic reticulum-mitochondria miscommunication is an early and causal trigger of hepatic insulin resistance and steatosis. J Hepatol 2022;77(3):710–722. doi:10.1016/j.jhep.2022.03.017, PMID:35358616.
[55] Kakisaka K, Sasaki A, Umemura A, Nikai H, Suzuki Y, Nishiya M, *et al*. High frequency and long persistency of ballooning hepatocyte were associated with glucose intolerance in patients with severe obesity. Sci Rep 2021;11(1):15392. doi:10.1038/s41598-021-94937-4, PMID:34321567.
[56] Mutel E, Abdul-Wahed A, Ramamonjisoa N, Stefanutti A, Houberdon I, Cavassila S, *et al*. Targeted deletion of liver glucose-6 phosphatase mimics glycogen storage disease type 1a including development of multiple adenomas. J Hepatol 2011;54(3):529–537. doi:10.1016/j.jhep.2010.08.014, PMID:21109326.
[57] Liu Q, Li J, Zhang W, Xiao C, Zhang S, Nian C, *et al*. Glycogen accumulation and phase separation drives liver tumor initiation. Cell 2021;184(22):5559–5576.e19. doi:10.1016/j.cell.2021.10.001, PMID:34678143.
[58] Shulman RG, Rothman DL. The Glycogen Shunt Maintains Glycolytic Homeostasis and the Warburg Effect in Cancer. Trends Cancer 2017;3(11):761–767. doi:10.1016/j.trecan.2017.09.007, PMID:29120752.
[59] Mazurek S, Boschek CB, Hugo F, Eigenbrodt E. Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol 2005;15(4):300–308. doi:10.1016/j.semcancer.2005.04.009, PMID:15908230.
[60] Ding DX, Wang Y, Yan W, Fu WN. MYCT1 alters the glycogen shunt by regulating selective translation of RACK1-mediated enzymes. iScience 2022;25(3):103955. doi:10.1016/j.isci.2022.103955, PMID:35281731.
[61] Guzman G, Chennuri R, Chan A, Rea B, Quintana A, Patel R, *et al*. Evidence for heightened hexokinase II immunoexpression in hepatocyte dysplasia and hepatocellular carcinoma. Dig Dis Sci 2015;60(2):420–426. doi:10.1007/s10620-014-3364-3, PMID:25381201.
[62] Allende DS, Gawrieh S, Cummings OW, Belt P, Wilson L, Van Natta M, *et al*. Glycogenosis is common in nonalcoholic fatty liver disease and is independently associated with ballooning, but lower steatosis and lower fibrosis. Liver Int 2021;41(5):996–1011. doi:10.1111/liv.14773, PMID:33354866.