HHS Public Access

Author manuscript

Biochem Biophys Res Commun. Author manuscript; available in PMC 2021 January 29.

Published in final edited form as:

Biochem Biophys Res Commun. 2020 January 29; 522(1): 1–7. doi:10.1016/j.bbrc.2019.11.061.

Activation of tumor-promoting pathways implicated in hepatocellular adenoma/carcinoma, a long-term complication of glycogen storage disease type Ia

Jun-Ho Cho[1,2], **Young Mok Lee**[1,2], **Seong-Ho Bae**[1], **Janice Y. Chou**[1,*]

[1]Section on Cellular Differentiation, Division of Translational Medicine, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA,

[2]Glycogen Storage Disease Program, Department of Pediatrics, University of Connecticut School of Medicine, Farmington, Connecticut, USA

Abstract

Hepatocellular adenoma/carcinoma (HCA/HCC) is a long-term complication of the metabolic disorder glycogen storage disease type Ia (GSD-Ia) deficient in glucose-6-phosphatase-α (G6PC or G6Pase-α). We have shown previously that hepatic G6Pase-α deficiency leads to autophagy impairment, mitochondrial dysfunction, enhanced glycolysis, and augmented hexose monophosphate shunt, all of which can contribute to hepatocarcinogenesis. However, the mechanism underlying HCA/HCC development in GSD-Ia remains unclear. We now show that G6Pase-α deficiency-mediated hepatic autophagy impairment leads to sustained accumulation of an autophagy-specific substrate p62 which can activate tumor-promoting pathways including nuclear factor erythroid 2-related factor 2 (Nrf2) and mammalian target of rapamycin complex 1 (mTORC1). Consistently, the HCA/HCC lesions developed in the G6Pase-α-deficient livers display marked accumulation of p62 aggregates and phosphorylated p62 along with activation of Nrf2 and mTORC1 signaling. Furthermore, the HCA/HCC lesions exhibit activation of additional oncogenic pathways, β-catenin and Yes-associated protein (YAP) which is implicated in autophagy impairment. Intriguingly, hepatic levels of glucose-6-phosphate and glycogen which are accumulated in the G6Pase-α-deficient livers were significantly lower in HCC than those in HCA. Conversely, compared to HCA, the HCC lesion display increased expression of many oncogenes and the M2 isoform of pyruvate kinase (PKM2), a glycolytic enzyme critical for aerobic glycolysis and tumorigenesis. Collectively, our data show that hepatic G6Pase-α-deficiency leads to persistent autophagy impairment and activation of multiple tumor-promoting pathways that contribute to HCA/HCC development in GSD-Ia.

*Correspondence should be addressed to: Janice Y. Chou, Building 10, Room 8N240C, NIH, 10 Center Drive, Bethesda, MD 20892-1830, Tel: 301-496-1094; Fax: 301-402-6035, chouja@mail.nih.gov.

Conflict of interest

The authors have declared no conflict of interest.

Keywords

Autophagy impairment; liver cancer; p62 accumulation; metabolism

1. Introduction

Glycogen storage disease type Ia (GSD-Ia, MIM232200) is caused by a deficiency in glucose-6-phosphatase-α (G6Pase-α or G6PC), a metabolic enzyme expressed mainly in the liver, kidney, and intestine [1]. G6Pase-α is a key enzyme for endogenous glucose production which catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose in the terminal step of glycogenolysis and gluconeogenesis [1]. GSD-Ia patients manifest impaired glucose homeostasis and a long-term manifestation of hepatocellular adenoma (HCA) that develops in 70–80% of GSD-Ia patients over 25 years of age [1]. In 10% of cases, HCA undergoes malignant transformation into hepatocellular carcinoma (HCC) [1].

We have shown that hepatic G6Pase-α deficiency leads to autophagy impairment, mitochondrial dysfunction, enhanced glycolysis, and augmented hexose monophosphate shunt (HMS) [2–4], all of which can contribute to hepatocarcinogenesis. A recent report have shown that chronic endoplasmic reticulum stress and downregulation of tumor suppressors are also implicated in hepatic tumorigenesis in GSD-Ia [5]. However, the mechanisms underlying HCA initiation and malignant transformation of HCA to HCC in GSD-Ia remain unclear. To study molecular mechanisms underlying HCA/HCC development in GSD-Ia, we generated a liver-specific *G6pc*-deficient (L-*G6pc*−/−) mouse line that develops HCA/HCC mimicking human GSD-Ia [2].

Autophagy is a recylcing process that involves lysosomal-mediated degradation of intracellular proteins and organelles [6]. Autophagy can supply energy and building blocks for metabolic pathway and also maintain cellular homemstasis by removing unfolded proteins and damages organelles [6]. Therefore, defective autophagy can lead to many pathological conditions including cancers [7]. Indeed, mice with liver-specific deletion of *Atg5* or *Atg7* develop HCA [8–10]. Autophagy impairment leads to the accumulation of p62, an autophagy-specific substrate [6]. Aberrant p62 accumulation can promote tumorigenesis via reactive oxygen species (ROS)-mediated genomic instability [11] and/or activation of tumor-promoting signaling pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2) and mammalian target of rapamycin complex 1 (mTORC1) [9, 12]. p62 accumulation can stabilize and activate Nrf2 by sequestering Kelch-like ECH-associated protein 1 (Keap1), an adaptor of the ubiquitin ligase for Nrf2 degradation [7]. p62 also promotes translocation of the mTORC1 complex to the lysosome, a crucial step for mTOR activation [13]. Notably, knockout of p62 in liver-specific *Atg7*−/− mice reduces hepatic tumor number and size [10], suggesting a critical role of p62 in hepatocarcinogenesis. Moreover, recent reports have shown that autophagy impairment is implicated in noncanonical activation of tumor-promoting pathways including β-catenin and Yes-associated protein (YAP) [14, 15].

In this study, we show that HCA/HCC lesions developed in the G6Pase-α-deficient livers display a marked increase in p62 aggregates and activation of Nrf2, mTORC1, β-catenin,

and YAP signaling pathways. These data suggest that sustained autophagy impairment caused by G6Pase-α deficiency contributes to activation of multiple tumor-promoting signaling pathways that can trigger HCA development and the transformation of HCA to HCC in GSD-Ia.

2. Materials and methods

2.1. Animals

All animal studies were conducted under an animal protocol approved by the Eunice Kennedy Shriver National Institute of Child Health and Human Development Animal Care and Use Committee. L-*G6pc*−/− and L-*G6pc*+/− mice were generated by tamoxifen-mediated excision of the *G6pc* exon 3 in 6-week-old $G6pc^{fx/fx}$.$SA^{creERT2/w}$ and $G6pc^{fx/w}$.$SA^{creERT2/w}$ mice, respectively, as previously described [2]. The phenotypes of L-*G6pc*+/+ and L-*G6pc*+/−are indistinguishable, thus both mice were used as controls. Liver samples were collected from mice at 12, 24, 53 and 78 WP (weeks post *G6pc* gene deletion) following a 6-hour fast.

2.2. Metabolites determination

The levels of lactate, G6P, and glucose in the samples were determined using the respective assay kit from BioVision (Mountain View, CA, USA). Hepatic glycogen levels were analyzed as described previously [16].

2.3. Quantitative real-time RT-PCR and Western-blot analysis

The mRNA expression was quantified by real-time RT-PCR using the TaqMan probes (Life Technologies, Carlsbad, CA, USA) in an Applied Biosystems QuantStudio 3 Real-Time PCR System (Foster City, CA, USA). Data were normalized to Rpl19 RNA or 18S rRNA. Western-blots were detected using the LI-COR Odyssey scanner (Li-Cor Biosciences, Lincoln, NE, USA). The antibodies used were β-actin (sc-47778) from Santa Cruz Biotechnology (Dallas, TX, USA); PKM2 (ab137791) from Abcam (Cambridge, MA, USA); p62 (NBP1–49956) from Novus Biologicals(Littleton, CO, USA); p-p62-S349 (#95697), Nrf2 (#12721), p-mTOR-S2448 (#5536), mTOR (#2983), p-4EBP-T37/46 (#2855), 4EBP (#9644), Dvl3 (#3218), non-phospho (active) β-catenin-S33/S37/T41 (#8814), YAP (#12395), p-YAP-S127 (#13008) from Cell Signaling Technology (Danvers, MA, USA).

2.4. Mutational analysis of exon 3 of β-catenin

RNAs were isolated from excised tumor nodules and the corresponding non-tumor tissues using the RNeasy Mini Kit (QIAGEN). cDNAs were synthesized using the High-capacity reverse transcription kit (Applied Biosystems). A 707-bp fragment containing exon 3 of the *Ctnnb1* (encoding β-catenin) gene that encompasses the sequence specific for GSK-3β phosphorylation was amplified using a primer pair: 5’-cgtggacaatggctactcaa-3’ (forward) and 5’-cttaaagatggccagcaagc-3’ (reverse). The amplified DNAs were purified and subjected to bidirectional DNA sequencing.

2.5. Immunohistochemical analysis

Liver or tumor samples were fixed in 10% neutral buffered formalin (Fisher Scientific, Grand Island, NY, USA), embedded in paraffin, and sectioned to 10 μm thickness. The sections were de-paraffinized by xylene and then incubated in antigen unmasking solution (Vector Laboratories, Burlingame, CA, USA) for 10 min at 100°C, and followed by incubation with 3% hydrogen peroxide solution in methanol to quench endogenous peroxidase activity in sections. The sections were then blocked with the Avidin/Biotin Blocking Kit (Vector Laboratories) and incubated with the antibody against anti-p62 (Novus Biologicals, NBP1–49956) or anti-β-catenin (Cell Signaling, #9582) and followed with the appropriate biotinylated secondary antibodies (Vector Laboratories). The resulting complexes in the sections were visualized with an ABC kit using the DAB Substrate (Vector Laboratories). The sections were also counterstained with hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) and visualized using a Zeiss Axioskop2 plus microscope equipped with 10X/0.45NA, 20X/0.5NA or 40X/0.75NA objectives (Carl Zeiss, Oberkochen, Germany).

2.6. Statistical analysis

The unpaired t test was performed using the GraphPad Prism Program, version 4 (San Diego, CA, USA). Values were considered statistically significant at $p < 0.05$.

3. Results

3.1. Persistent hepatic accumulation of p62 in the L-G6pc−/−mice

We have shown previously that G6Pase-α deficiency leads to hepatic autophagy impairment and mitochondrial dysfunction [2, 3]. We now show that hepatic accumulation of p62, an autophagy-specific substrate persisted in the L-*G6pc*−/− mice from the pre-tumor stage (12 WP and 24 WP) to the tumor-developing stage (53 WP) (Fig. 1A). Compared to the controls, hepatic levels of the phosphorylated p62 (p-p62) was increased primarily at the tumor-developing stage of 53 WP (Fig. 1A). We have shown that the G6Pase-α-deficient livers progressively accumulate damaged mitochondria [3], a potential source of ROS which can induce p62 phosphorylation [17]. Moreover, p-p62 has an increased affinity to Keap1 that is responsible for Nrf2 degradation, leading to robust activation of Nrf2 signaling [17]. Consistently, the livers of L-*G6pc*−/− mice at the tumor-developing stage displayed increased levels of Nrf2, compared to age-matched controls (Fig. 1A). The marked accumulation of p62 is generally followed by the formation of p62 aggregates [18], which are frequently observed in human HCC [9, 12]. Intriguingly, p62 aggregates were present mainly in the livers of L-*G6pc*−/− mice at the tumor-developing stage, while they were barely detected in L-*G6pc*−/−mice at the pre-tumor stage and control mice (Fig. 1B).

3.2. Accumulation of p62 aggregates and activation of Nrf2 and mTORC1 signaling in the HCA/HCC lesions

Aberrant p62 accumulation results in p62 aggregates and activates tumor-promoting pathways, Nrf2 and mTORC1 [9, 19]. Immunofluorescence analysis revealed the presence of p62 aggregates in the HCA/HCC lesions of the L-*G6pc*−/− mice (Fig. 2A). Western-blot analysis confirmed the marked accumulation of p62 and p-p62 in the tumor lesions (Fig.

2B). As expected, the HCA/HCC lesions displayed elevated protein levels of Nrf2 (Fig. 2B) along with increased expression of Nrf2 target genes, NAD(P)H dehydrogenase quinone (*Nqo1*) and glutamate-cysteine ligase catalytic subunit (*Gclc*) (Fig. 2C). Studies have shown that the activation status of mTORC1 signaling is dictated by p-mTOR-S2448 [20]. Compared to non-tumor liver tissues, levels of p-mTOR-S2448 were markedly increased in the HCA/HCC lesions of L-*G6pc*−/− mice (Fig. 2B). In tumor lesions, activation of mTORC1 signaling was further confirmed by increased phosphorylation of eIF4E Binding Protein (4EBP), a target substrate of mTORC1 (Fig. 2B). Collectively, these results show that the tumor lesions in L*G6pc*−/− mice display accumulation of p62 aggregates, and activation of Nrf2 and mTORC1 signaling which play important roles in cancer cells survival and proliferation, respectively.

3.3. Activation of β-catenin and YAP signaling in the HCA/HCC lesions

To delineate the roles of defective autophagy in HCA/HCC development, we examined the other tumorigenic pathways activated by autophagy deficiency. In many cancers including HCC, β-catenin mutations and the resulting β-catenin protein stabilization lead to activation of Wnt signaling [21]. Moreover, HCAs developed in GSD-Ia patients exhibit higher frequency of β-catenin-mutated HCA which display a higher risk of malignant transformation to HCC, compared to HCA in the general population [22]. In this study, none of the HCA/HCC lesions analyzed harbored activating β-catenin mutations except one HCA nodule harboring two missense mutations in codon Gly-34 of β-catenin which have been reported in HCC [21] (Supplemental Data. 1). However, the Wnt/β-catenin pathway can also be negatively regulated by autophagy-mediated degradation of the Dishevelled (Dvl) protein [14], which, when activated by Wnt, stabilizes β-catenin [21]. We therefore examined whether β-catenin activation can be mediated by hepatic accumulations of the Dvl protein in the absence of β-catenin mutation. In L-*G6pc*−/− mice, levels of Dvl3 were significantly elevated in the HCA/HCC nodules, compared to the non-tumor liver tissues (Fig. 3A). Consistent with Dvl3 accumulation, levels of active β-catenin were elevated in tumor lesions along with increased expression of its target gene, c-Myc (Fig. 3B). Immunohistochemical analysis confirmed the increased accumulation of β-catenin in the HCA/HCC nodules (Fig. 3C).

Next, we examined the expression of YAP, a transcriptional co-activator in the Hippo/YAP pathway [22], since YAP promotes hepatocarcinogenesis in autophagy-impaired livers [15]. Western-blot analysis showed that in HCA/HCC lesions, protein levels of YAP were significantly increased in parallel to those of Dvl3 (Fig. 3A). Moreover, mRNA levels of YAP targeted genes, including cysteine-rich angiogenic inducer 61 (*Cyr61*) and baculoviral inhibitor of apoptosis repeat-containing 5 (*Birc5*) were increased in tumor lesions (Fig. 3B). However, YAP transcripts were similar between tumor and non-tumor tissues (Fig. 3B), ruling out its transcriptional regulation. In the YAP/Hippo pathway, protein levels of YAP are negatively regulated by phosphorylation and subsequent proteasomal degradation of YAP [22]. However, compared to the non-tumor liver tissues, levels of the phosphorylated YAP were unaltered in the HCA/HCC nodules (Fig. 3A), suggesting that alternative degradation pathway involves in the increase in YAP. Since YAP is a target protein of

autophagy-mediated degradation [15], the defective autophagy provides an explanation why YAP signaling is activated in HCA/HCC lesions in L-*G6pc*−/− mice.

3.4. Augmented oncogenes expression and reduced G6P/glycogen storage in HCC lesions

We then sought to understand potential mechanisms underlying the transformation of HCA to HCC by examining differential oncogenes expression and metabolites between HCA and HCC lesions. We showed that the expression of Nrf2, YAP, and β-catenin target genes were markedly increased in both HCA and HCC nodules (Fig. 4A). However, the increases were more prominent in the HCC lesions, compared to HCA lesions (Fig. 4A). G6Pase-α deficiency lead to increased hepatic accumulation of G6P, glycogen, and lactate along with reduced hepatic levels of glucose [1]. Moreover, we have shown previously that the HCA/HCC lesions in L-*G6pc*−/− mice display increased expression of the M2 isoform of pyruvate kinase (PKM2) critical for aerobic glycolysis and tumorigenesis [4]. We now show that upregulation of PKM2 was observed primarily in the HCC lesions (Fig. 4B), while lactate levels were unchanged in HCA/HCC lesions, compared to corresponding non-tumor liver tissues (Fig. 4C). Compared to corresponding non-tumor liver tissues, glucose levels were decreased in both HCA and HCC lesions, although the decrease in HCA lesions did not reach statistical significance (Fig. 4D). However, G6P levels were decreased exclusively in the HCC lesions (Fig. 4D), while glycogen levels were decreased in both HCA and HCC lesions, compared to respective non-tumor liver tissues (Fig. 4E). Intriguingly, levels of G6P (Fig. 4D) and glycogen (Fig. 4E) were significantly reduced in HCC lesions than HCA lesions, raising the possibility that either consumption or production of these metabolites differs between HCA and HCC. Collectively, the data suggest that increased expression of oncogenes and PKM2, and differential metabolism including reduced G6P and glycogen storage in HCC lesions may play a role in the transformation of HCA to HCC.

4. Discussion

GSD-Ia patients manifest the life-threatening fasting hypoglycemia and a longer-term complication of HCA/HCC of unknown etiology. We have shown previously that hepatic G6Pase-α deficiency leads to autophagy impairment, mitochondrial dysfunction, enhanced glycolysis, and augmented HMS [2–4], all of which can contribute to hepatic tumorigenesis. Using L-*G6pc*−/− mice, we now show that sustained autophagy impairment by G6Pase-α deficiency is implicated in activation of multiple tumor-promoting pathways including Nrf2, mTORC1, β-catenin, and YAP which can trigger HCA/HCC development in GSD-Ia.

A recent study has shown that p62 accumulation during preneoplasia induces HCC development in mice via activation of Nrf2 and mTORC1 signaling [23]. Nrf2 promotes tumor cells survival by inducing the expression of cytoprotective and detoxifying genes [7], and mTORC1 plays important roles in cancer growth, proliferation and metabolism [24]. Interestingly, Nrf2 can induce the expression of p62 [25], and the activated mTORC1 can inhibit autophagy, resulting in p62 accumulation [24]. Thus, in L-*G6pc*−/− mice, aberrant hepatic p62 accumulation can activate Nrf2 and mTORC1 signaling which, in turn, stimulate more p62 accumulation, establishing a self-amplifying feedback loop in p62-Nrf2-mTORC1

signaling. The HCA lesions found in human GSD-Ia patients harbor Mallory-Denk bodies [26], which are protein aggregates primarily composed of p62, keratin, and ubiquitin [27]. Consistently, the HCA/HCC lesions developed in the G6Pase-α-deficient livers exhibited accumulation of p62 aggregates along with activation of Nrf2, mTORC1, β-catenin, and YAP signaling.

It has been suggested that malignant cancers like HCC require autophagy to obtain sufficient energy and building blocks for rapid proliferation and/or to survive in the stressful tumor microenvironments [7, 28]. Thus, autophagy-deficient mice with liver-specific deletion of *Atg5* or *Atg7* develop only HCA but not HCC [12–14]. In contrast, autophagy-impaired L-*G6pc*−/− mice developed HCC as well as HCA. One explanation is a reactivation of impaired autophagy during malignant transformation of HCA to HCC in L-*G6pc*−/− mice. However, both HCA and HCC lesions developed in L-*G6pc*−/− mice display signs of autophagy impairment evident by p62 accumulation and mTORC1 signaling activation, ruling out this possibility. Another possibility lies in the alternative sources of energy present in L-*G6pc*−/−mice. Tumors can use metabolites including glycogen [29] and extracellular lactate [30] as alternative energy sources. The marked increases in the levels of hepatic glycogen, lactate and/or G6P by hepatic G6Pase-α deficiency may explain HCC development in the presence of autophagy impairment. Thus, it is noteworthy that levels of G6P and glycogen in HCC nodules were significantly lower than those in HCA nodules.

In summary, we show that hepatic G6Pase-α deficiency leads to sustained autophagy impairment which can initiate tumor development in L-*G6pc*−/− mice via activation of Nrf2, mTORC1, β-catenin, and YAP signaling. Furthermore, mitochondrial dysfunction and metabolic alterations caused by hepatic G6Pase-α deficiency provide additional signals to promote HCA development and its transformation to HCC. Our study suggests that the strategy to normalize autophagy impairment may be a worthy avenue to slow down or prevent HCA/HCC development in GSD-Ia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We thank Dr. Pierre Chambon for the gist of the AlbCreERT2 mice. This research was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, and The Children's Fund for Glycogen Storage Disease Research.

Abbreviations

HCA/HCC	Hepatocellular adenoma/carcinoma
GSD-Ia	glycogen storage disease type Ia
G6Pase-α	glucose-6-phosphatase-α
Nrf2	nuclear factor erythroid 2-related factor 2

mTORC1	mammalian target of rapamycin complex 1
YAP	Yes-associated protein
PKM2	the M2 isoform of pyruvate kinase
G6P	glucose-6-phosphate
ROS	reactive oxygen species
Keap1	Kelch-like ECH-associated protein 1
H&E	Hematoxylin and eosin
Nqo1	NAD(P)H dehydrogenase quinone
Gclc	glutamate-cysteine ligase catalytic subunit
4EBP	eIF4E Binding Protein
Dvl	Dishevelled
Cyr 61	cysteine-rich angiogenic inducer 61
Birc5	baculoviral inhibitor of apoptosis repeat-containing 5

References

[1]. Chou JY, Jun HS, Mansfield BC, Glycogen storage disease type I and G6Pase-beta deficiency: etiology and therapy, Nat Rev Endocrinol, 6 (2010) 676–688. [PubMed: 20975743]

[2]. Cho JH, Kim GY, Pan CJ, Anduaga J, Choi EJ, Mansfield BC, Chou JY, Downregulation of SIRT1 signaling underlies hepatic autophagy impairment in glycogen storage disease type Ia, PLoS genetics, 13 (2017) e1006819. [PubMed: 28558013]

[3]. Cho JH, Kim GY, Mansfield BC, Chou JY, Sirtuin signaling controls mitochondrial function in glycogen storage disease type Ia, Journal of inherited metabolic disease, 41 (2018) 997–1006.

[4]. Cho JH, Kim GY, Mansfield BC, Chou JY, Hepatic glucose-6-phosphatase-alpha deficiency leads to metabolic reprogramming in glycogen storage disease type Ia, Biochemical and biophysical research communications, 498 (2018) 925–931. [PubMed: 29545180]

[5]. Gjorgjieva M, Calderaro J, Monteillet L, Silva M, Raffin M, Brevet M, Romestaing C, Roussel D, Zucman-Rossi J, Mithieux G, Rajas F, Dietary exacerbation of metabolic stress leads to accelerated hepatic carcinogenesis in glycogen storage disease type Ia, J Hepatol, 69 (2018) 1074–1087. [PubMed: 30193922]

[6]. Mizushima N, Komatsu M, Autophagy: renovation of cells and tissues, Cell, 147 (2011) 728–741. [PubMed: 22078875]

[7]. White E, Deconvoluting the context-dependent role for autophagy in cancer, Nat Rev Cancer, 12 (2012) 401–410. [PubMed: 22534666]

[8]. Ni HM, Woolbright BL, Williams J, Copple B, Cui W, Luyendyk JP, Jaeschke H, Ding WX, Nrf2 promotes the development of fibrosis and tumorigenesis in mice with defective hepatic autophagy, J Hepatol, 61 (2014) 617–625. [PubMed: 24815875]

[9]. Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M, Lee MS, Tanaka K, Komatsu M, Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells, J Cell Biol, 193 (2011) 275–284. [PubMed: 21482715]

[10]. Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N, Autophagy-deficient mice develop multiple liver tumors, Genes Dev, 25 (2011) 795–800. [PubMed: 21498569]

[11]. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, Bray K, Reddy A, Bhanot G, Gelinas C, Dipaola RS, Karantza-Wadsworth V, White E, Autophagy suppresses tumorigenesis through elimination of p62, Cell, 137 (2009) 1062–1075. [PubMed: 19524509]

[12]. Moscat J, Diaz-Meco MT, p62 at the crossroads of autophagy, apoptosis, and cancer, Cell, 137 (2009) 1001–1004. [PubMed: 19524504]

[13]. Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, Porollo A, Hansen M, Moscat J, Diaz-Meco MT, p62 is a key regulator of nutrient sensing in the mTORC1 pathway, Molecular cell, 44 (2011) 134–146. [PubMed: 21981924]

[14]. Gao C, Cao W, Bao L, Zuo W, Xie G, Cai T, Fu W, Zhang J, Wu W, Zhang X, Chen YG, Autophagy negatively regulates Wnt signalling by promoting Dishevelled degradation, Nat Cell Biol, 12 (2010) 781–790. [PubMed: 20639871]

[15]. Lee YA, Noon LA, Akat KM, Ybanez MD, Lee TF, Berres ML, Fujiwara N, Goossens N, Chou HI, Parvin-Nejad FP, Khambu B, Kramer EGM, Gordon R, Pfleger C, Germain D, John GR, Campbell KN, Yue Z, Yin XM, Cuervo AM, Czaja MJ, Fiel MI, Hoshida Y, Friedman SL, Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap, Nat Commun, 9 (2018) 4962. [PubMed: 30470740]

[16]. Yiu WH, Lee YM, Peng WT, Pan CJ, Mead PA, Mansfield BC, Chou JY, Complete normalization of hepatic G6PC deficiency in murine glycogen storage disease type Ia using gene therapy, Mol Ther, 18 (2010) 1076–1084. [PubMed: 20389290]

[17]. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, Saito T, Yang Y, Kouno T, Fukutomi T, Hoshii T, Hirao A, Takagi K, Mizushima T, Motohashi H, Lee MS, Yoshimori T, Tanaka K, Yamamoto M, Komatsu M, Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy, Molecular cell, 51 (2013) 618–631. [PubMed: 24011591]

[18]. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E, Tanaka K, Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice, Cell, 131 (2007) 1149–1163. [PubMed: 18083104]

[19]. Puissant A, Fenouille N, Auberger P, When autophagy meets cancer through p62/SQSTM1, Am J Cancer Res, 2 (2012) 397–413. [PubMed: 22860231]

[20]. Chiang GG, Abraham RT, Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase, The Journal of biological chemistry, 280 (2005) 25485–25490. [PubMed: 15899889]

[21]. Polakis P, Wnt signaling and cancer, Genes Dev, 14 (2000) 1837–1851. [PubMed: 10921899]

[22]. Piccolo S, Dupont S, Cordenonsi M, The biology of YAP/TAZ: hippo signaling and beyond, Physiol Rev, 94 (2014) 1287–1312. [PubMed: 25287865]

[23]. Umemura A, He F, Taniguchi K, Nakagawa H, Yamachika S, Font-Burgada J, Zhong Z, Subramaniam S, Raghunandan S, Duran A, Linares JF, Reina-Campos M, Umemura S, Valasek MA, Seki E, Yamaguchi K, Koike K, Itoh Y, Diaz-Meco MT, Moscat J, Karin M, p62, Upregulated during Preneoplasia, Induces Hepatocellular Carcinogenesis by Maintaining Survival of Stressed HCC-Initiating Cells, Cancer cell, 29 (2016) 935–948. [PubMed: 27211490]

[24]. Laplante M, Sabatini DM, mTOR signaling in growth control and disease, Cell, 149 (2012) 274–293. [PubMed: 22500797]

[25]. Jain A, Lamark T, Sjottem E, Larsen KB, Awuh JA, Overvatn A, McMahon M, Hayes JD, Johansen T, p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription, J Biol Chem, 285 (2010) 22576–22591. [PubMed: 20452972]

[26]. Bianchi L, Glycogen storage disease I and hepatocellular tumours, European journal of pediatrics, 152 Suppl 1 (1993) S63–70. [PubMed: 8391447]

[27]. Zatloukal K, French SW, Stumptner C, Strnad P, Harada M, Toivola DM, Cadrin M, Omary MB, From Mallory to Mallory-Denk bodies: what, how and why?, Experimental cell research, 313 (2007) 2033–2049. [PubMed: 17531973]

[28]. Cui J, Gong Z, Shen HM, The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets, Biochim Biophys Acta, 1836 (2013) 15–26. [PubMed: 23428608]

[29]. Favaro E, Bensaad K, Chong MG, Tennant DA, Ferguson DJ, Snell C, Steers G, Turley H, Li JL, Gunther UL, Buffa FM, McIntyre A, Harris AL, Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells, Cell Metab 16 (2012) 751–764. [PubMed: 23177934]

[30]. Doherty JR, Cleveland JL, Targeting lactate metabolism for cancer therapeutics, J Clin Invest 123 (2013) 3685–3692. [PubMed: 23999443]

Highlights

- Hepatic G6Pase-α deficiency leads to sustained autophagy impairment and p62 accumulation.
- HCA/HCC in murine GSD-Ia display marked p62 aggregates and p62 phosphorylation.
- Nrf2, mTORC1, β-catenin, and YAP pathways are activated in HCA/HCC in murine GSD-Ia.
- HCC and HCA in murine GSD-Ia exhibit differential metabolism and oncogenes expression.

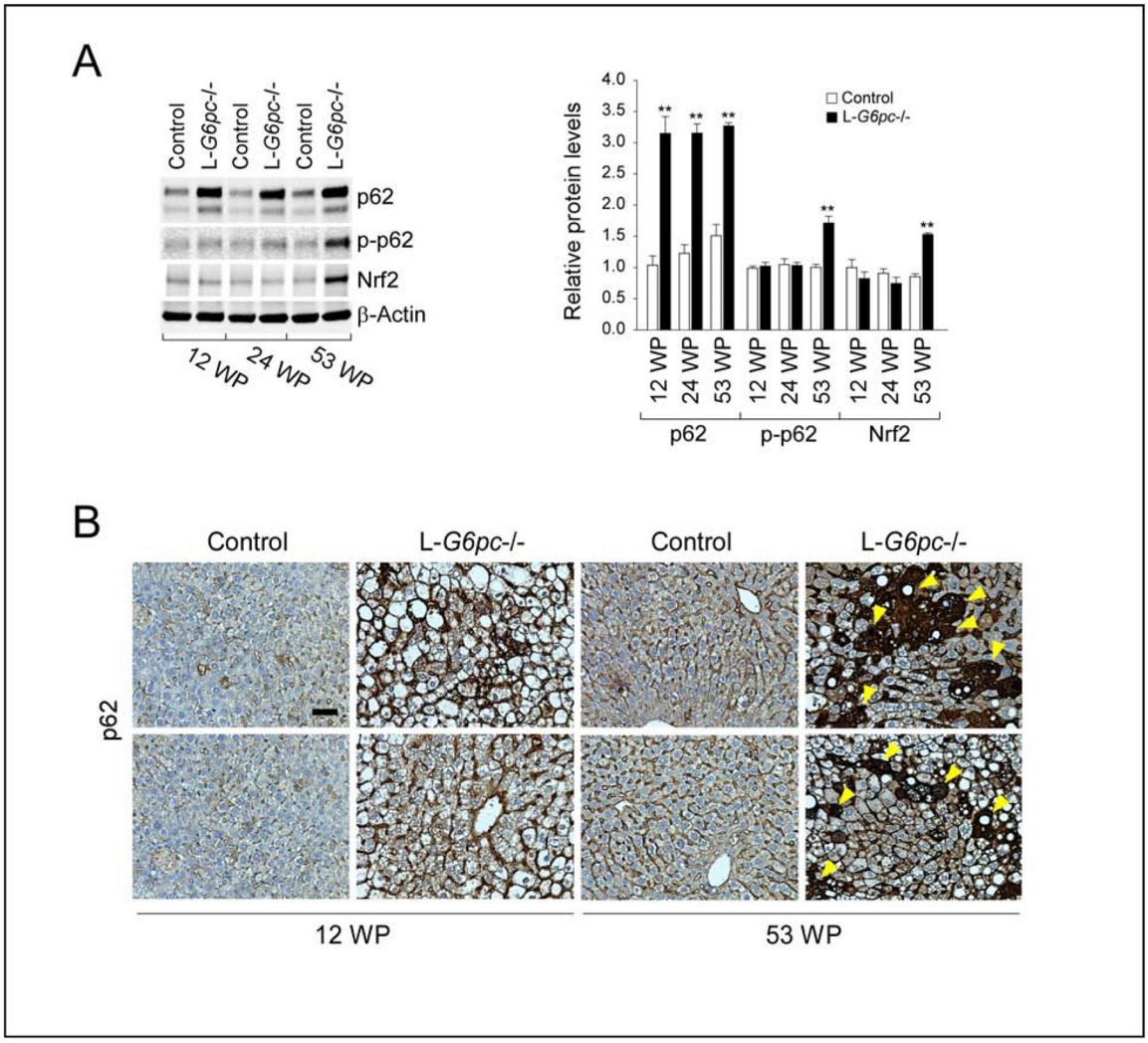

Fig. 1.

Sustained hepatic p62 accumulation in L-*G6pc*−/− mice. (A) Western-blots for the indicated hepatic proteins in control and L-*G6pc*−/− mice at 12, 24 and 53 WP (weeks post *G6pc* gene deletion), and quantification by densitometry (n = 3). (B) Immunohistochemical analysis of hepatic p62 in control and L-*G6pc*−/− mouse at 12 and 53 WP. Arrowheads indicate the cells containing p62 aggregates. Scale bar, 50 μm. Data represent the mean ± SEM. ** $P <$ 0.005.

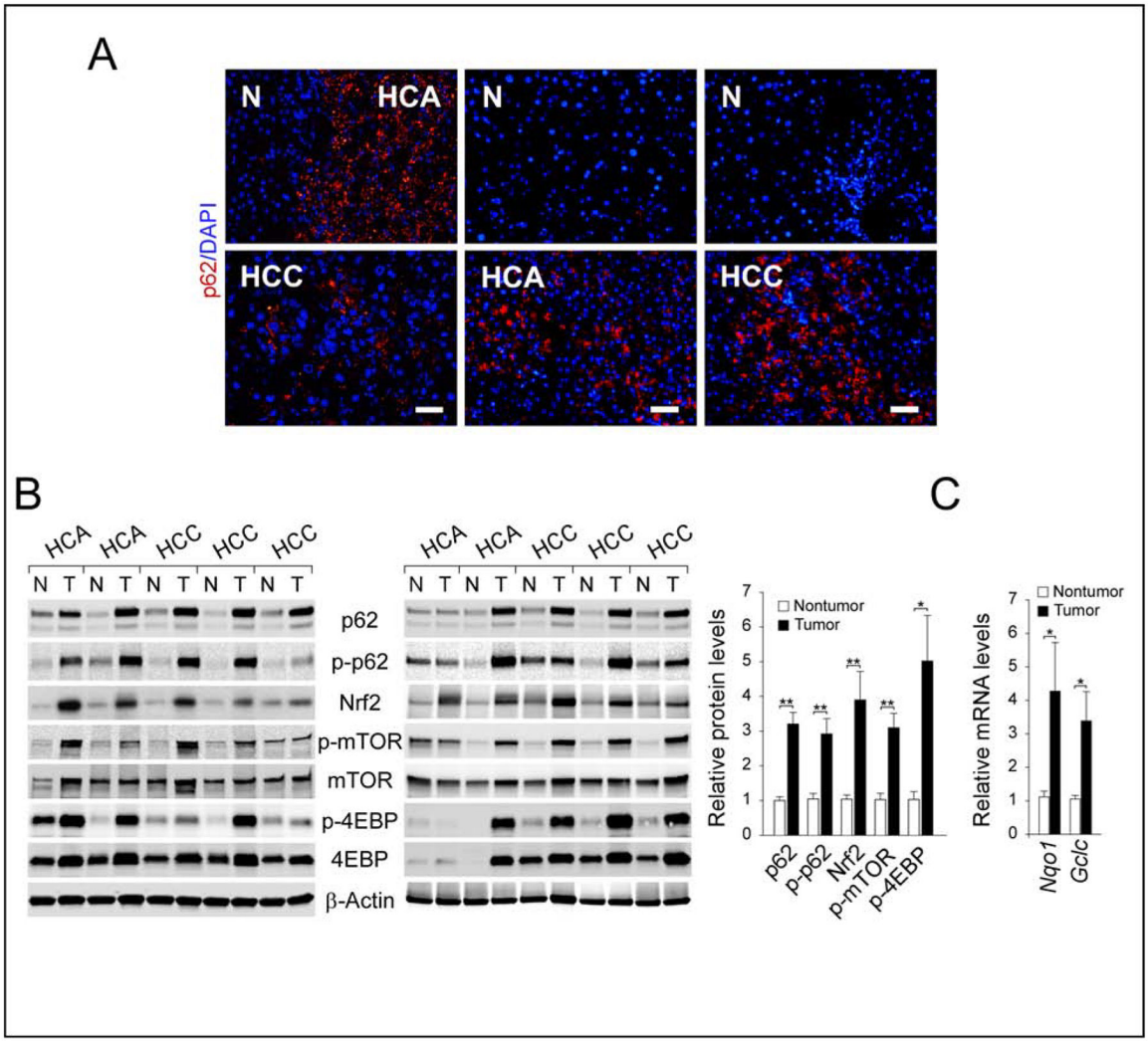

Fig. 2.

p62 aggregates and activated Nrf2 and mTORC1 signaling in HCA/HCC lesions. (A) Immunofluorescence analysis of p62 (red) and DAPI-stained nuclei (blue) in non-tumor (N) liver regions, HCA or HCC lesions of L-*G6pc*−/− mouse. Scale bar, 50 μm. (B) Western-blots for the indicated proteins in tumor (T) and corresponding non-tumor (N) liver region of L-*G6pc*−/− mice, and quantification by densitometry (n = 10). (C) Quantification of mRNA for *Nqo1* and *Gclc* of non-tumor and tumor regions by real-time RT-PCR (n = 10). Data represent the mean ± SEM. **P* < 0.05, ***P* < 0.005.

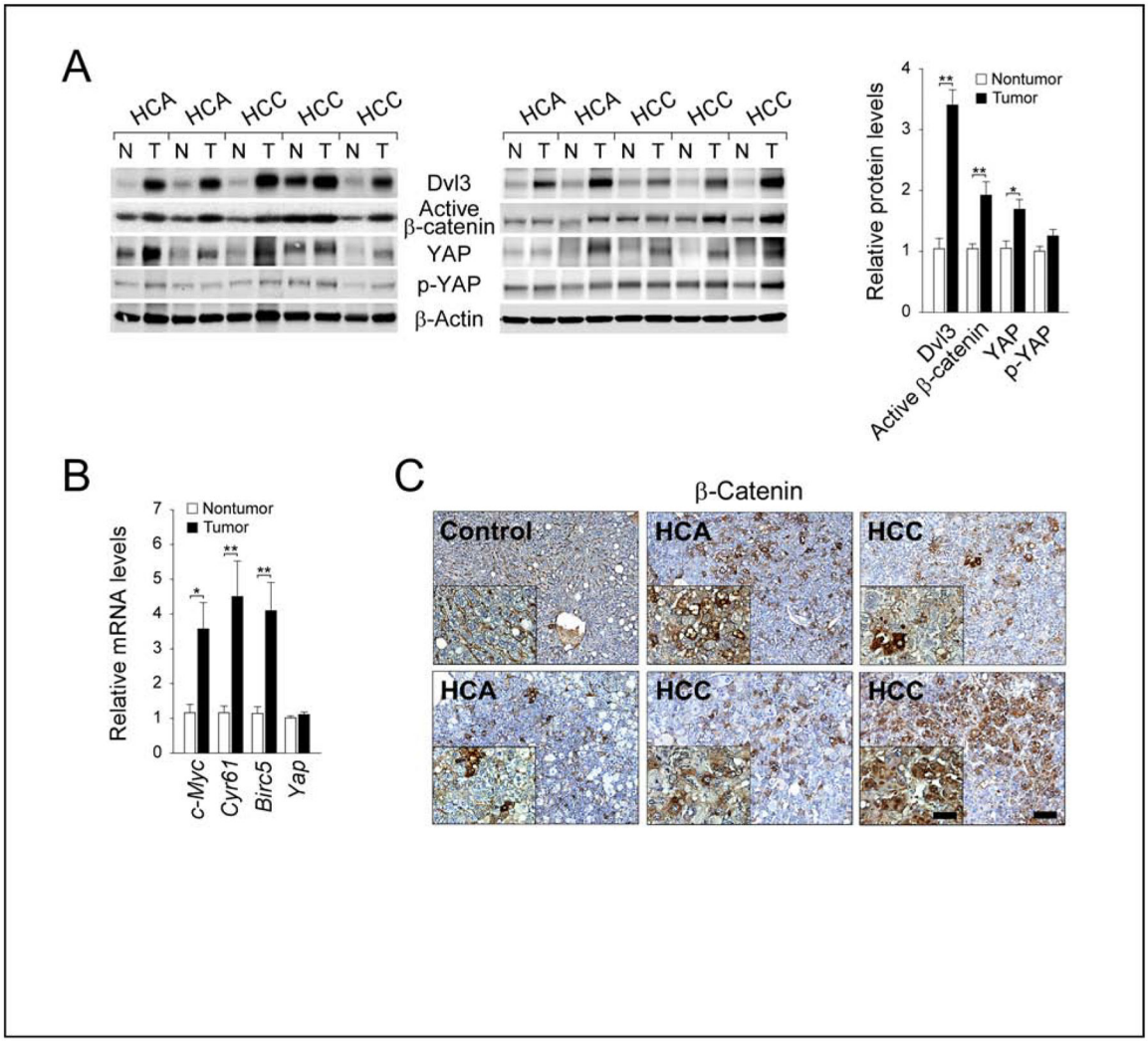

Fig. 3.

Activated β-catenin and YAP pathways in HCA/HCC lesions. (A) Western-blots for the indicated proteins in tumor (T) and corresponding non-tumor (N) liver region of L-*G6pc*−/− mice, and quantification by densitometry (n = 10). (B) Quantification of mRNA for *c-Myc*, *Cyr61*, *Birc5*, and *Yap* of non-tumor and tumor regions by real-time RT-PCR (n = 10). (C) Immunohistochemical analysis of β-catenin in control and HCA/HCC-bearing L-*G6pc*−/−mice. Scale bar, 100 μm. The insets represent higher magnification views. Scale bar, 25 μm. Data represent the mean ± SEM. **P* < 0.05, ***P* < 0.005.

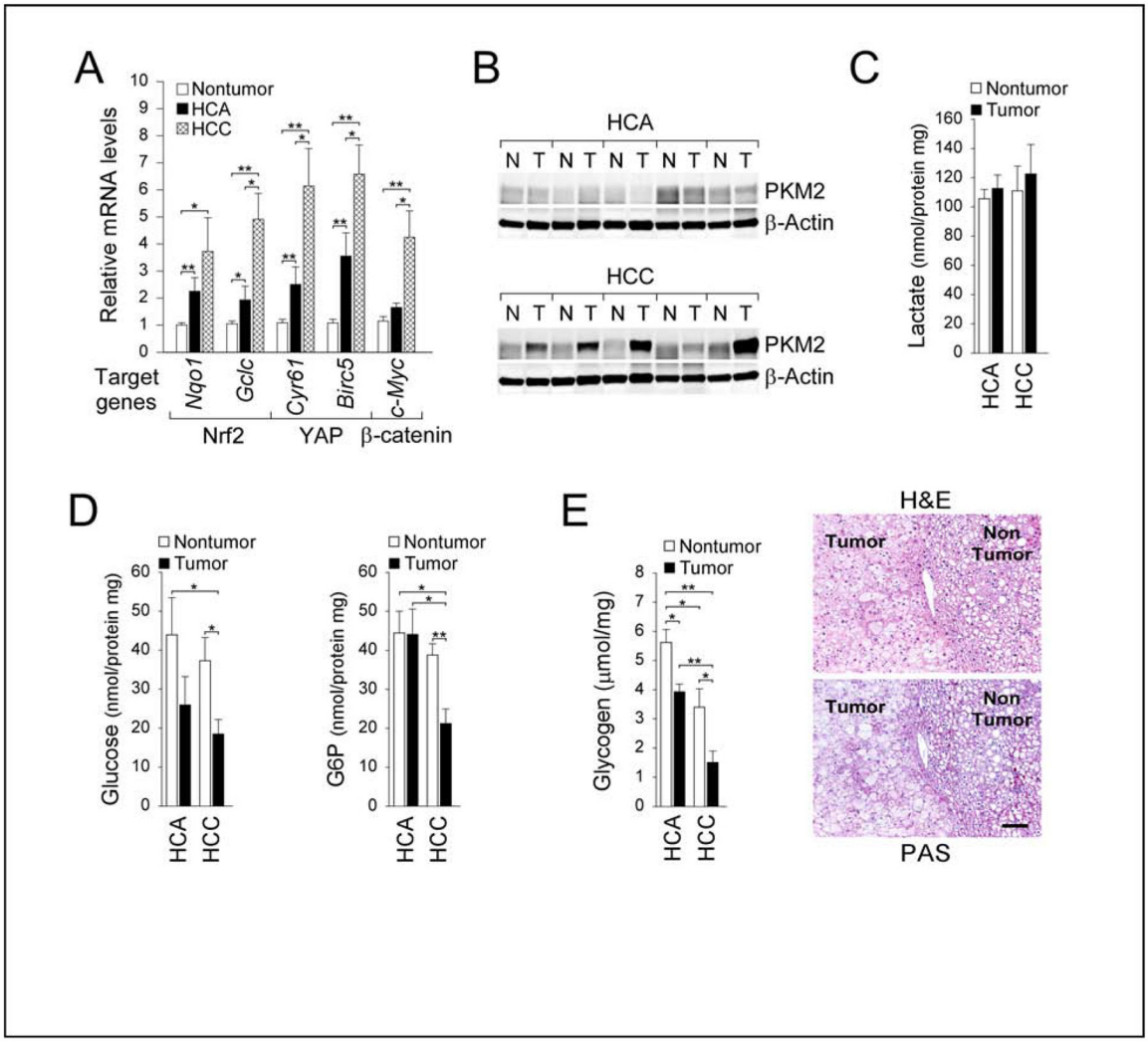

Fig. 4.

Augmented oncogenes expression and differential metabolism in HCC lesions. (A) Quantification of mRNA for *Nqo1, Gclc, Cyr61, Birc5*, and *c-Myc* of non-tumor (n = 10), HCA (n = 6), and HCC (n = 10) regions by real-time RT-PCR. (B) Western-blots for hepatic PKM2 and β-actin in the non-tumor (N) liver tissues and tumor (T) lesions of the L-*G6pc*−/− mice. (C) Lactate levels in HCA and corresponding non-tumor regions (n = 7), and HCC and corresponding non-tumor regions (n = 10). (D) Hepatic levels of glucose and G6P in HCA and corresponding non-tumor regions (n = 7), and HCC and corresponding non-tumor regions (n = 10). (E) Hepatic glycogen levels in HCA and corresponding non-tumor regions (n = 5), and HCC and corresponding non-tumor regions (n = 5). H&E and Periodic acid-Schiff (PAS) staining for tumor and the corresponding non-tumor region. Scale bar, 100 μm. Data represent the mean ± SEM. **P* < 0.05, ***P* < 0.005.