AMPK and Akt/mTOR signalling pathways participate in glucose-mediated regulation of hepatitis B virus replication and cellular autophagy

Xueyu Wang | Yong Lin | Thekla Kemper | Jieliang Chen | Zhenghong Yuan | Shi Liu | Ying Zhu | Ruth Broering | Mengji Lu

1 Institute of Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
2 Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), Shanghai Medical College, Fudan University, Shanghai, China
3 State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China
4 Department of Gastroenterology and Hepatology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

Abstract
A growing consensus indicates that host metabolism plays a vital role in viral infections. Hepatitis B virus (HBV) infection occurs in hepatocytes with active glucose metabolism and may be regulated by cellular metabolism. We addressed the question whether and how glucose regulates HBV replication in hepatocytes. The low glucose concentration at 5 mM significantly promoted HBV replication via enhanced transcription and autophagy when compared with higher glucose concentrations (10 and 25 mM). At low glucose concentration, AMPK activity was increased and led to ULK1 phosphorylation at Ser 555 and LC3-II accumulation. By contrast, the mTOR pathway was activated by high glucose concentrations, resulting in reduced HBV replication. mTOR inhibition by rapamycin reversed negative effects of high glucose concentrations on HBV replication, suggesting that low glucose concentration promotes HBV replication by stimulating the AMPK/mTOR/ULK1-autophagy axis. Consistently, we found that glucose transporters inhibition using phloretin also enhanced HBV replication via increased AMPK/mTOR/ULK1-induced autophagy. Surprisingly, the glucose analogue 2-deoxy-D-glucose reduced HBV replication through activating the Akt/mTOR signalling pathway also at the low glucose concentrations. Our study reveals that glucose is an important factor for the HBV life cycle by regulating HBV transcription and posttranscriptional steps of HBV replication via cellular autophagy.

KEYWORDS
hepatitis B virus, glucose, 2-DG, autophagy, AMPK, mTOR

1 | INTRODUCTION

Hepatitis B virus (HBV) infection is still a major global public health problem. According to the recent estimation of the WHO, there are approximately 257 million chronically HBV-infected patients...
worldwide. Patients with chronic HBV infection (CHB) often suffer from severe liver diseases including fibrosis, cirrhosis, and hepatocellular carcinoma (Ganem & Prince, 2004). The intrinsic mechanisms of liver disease progression in CHB are under active investigation. Nevertheless, there is no treatment available for effective eradication of CHB yet. Therefore, it is essential to further investigate the complex host-HBV interaction to gain a deeper understanding of the mechanisms of HBV pathogenesis and to identify new therapeutic targets.

Glucose is the most important nutrition and energy source for organisms and is the raw material for glycolysis. To be utilised, glucose is taken up via glucose transporters (GLUTs) and converted to glucose 6-phosphate by hexokinases (HKs), the first and rate-limiting step in glycolysis (Adeva-Andany, Perez-Felpete, Fernandez-Fernandez, Donapetry-Garcia, & Pazos-Garcia, 2016). Glucose 6-phosphate may enter various metabolic pathways, including glycolysis, glycogen synthesis, the hexosamine pathway, the pentose phosphate pathway, and oxidative routes (DeBerardinis & Chandel, 2016). Glycolysis is an essential cellular pathway in all cell types. During this process, glucose is metabolized to pyruvate, with subsequent releases of two molecules of adenosine triphosphate (ATP) and two nicotinamide adenine dinucleotides. Then, pyruvate is reduced to lactate under anaerobic conditions. However, pyruvate may transfer to mitochondria and be oxidized to produce acetylcoenzyme A under aerobic conditions, sustaining the tricarboxylic acid cycle. Several small molecule inhibitors can be used to interfere with glucose uptake and glycolysis. Phloretin is a phenolic compound found in apples (Lee, Kim, Kim, Lee, & Lee, 2003) and strawberries (Hilt et al., 2003) and is an inhibitor of GLUTs. A glucose analogue, 2-deoxy-D-glucose (2-DG), has a 2-hydroxy group substituted by hydrogen and can also enter the cell through GLUTs. However, 2-DG is phosphorylated to 2-DG-6-P by HKs, which in turn blocks HK activity and inhibits glycolysis.

Cellular energy metabolism can globally alter other processes, such as transcription and autophagy (Lindqvist, Tandoc, Topisirovic, & Furic, 2018). Glucose metabolism directly influences the abundance of related transcription factors as well as downstream gene expression (Metallo & Vander Heiden, 2013; Vaulont, Vasseur-Cognet, & Kahn, 2000). Under nutrient-rich conditions, mTORC1 is activated to support cell growth and to block autophagy via inhibition of ULK1. Low cellular energy metabolism activates AMPK that subsequently phosphorylates ULK1 on amino acid residue Ser 555, thereby inducing autophagy. Glycolysis inhibition by 2-DG has indirect impact on many cellular pathways. As an example, glucose deprivation or glycolysis inhibition by 2-DG results in decreased ATP levels and increased adenosine monophosphate (AMP) levels in cells. An elevated AMP/ATP ratio leads to AMPK activation and triggers downstream pathways.

In response to viral infections, glycolysis may be increased or decreased, with beneficial effects for viruses, for example, in the case of herpes simplex virus 1 and adenovirus infections (Abrantes et al., 2012; Thai et al., 2014). Studies found that HBV infection modulated host liver metabolic pathways, resulting in upregulation of glucose metabolism (e.g., gluconeogenesis, aerobic oxidation of glucose, and pentose phosphate pathway; Liu et al., 2015; Shin et al., 2011) and lipid metabolism (e.g., fatty acids, phospholipids, and cholesterol biosynthesis; Chen, Liang, Ou, Goldstein, & Brown, 2004; M. D. Wang et al., 2016; Y. L. Wu et al., 2016; Yang et al., 2008). These reports suggest a complex relationship between HBV infection and metabolic changes in hepatocytes. Nevertheless, the impact of the changes in hepatic metabolisms on HBV replication has not been studied so far in detail.

Therefore, we aimed to provide experimental evidence for the hypothesis that glucose metabolism regulates HBV replication. Indeed, the low glucose concentration in cell cultures and inhibition of GLUTs led to activation of AMPK–mTOR–ULK1–autophagy axis in hepatocytes. In recent years, many studies demonstrated that HBV replication depended on cellular autophagy, and the AMPK–Akt/mTOR–ULK1-induced autophagy pathway significantly regulates HBV replication (Lin et al., 2017; Lin et al., 2019; J. Wang et al., 2019; Xie et al., 2016). In the present study, we found that HBV replication and gene expression were modulated by varying glucose concentrations in the cell culture medium. Treatment with 2-DG decreased glycolysis and activated the Akt/mTOR pathway, resulting in the inhibition of HBV replication, even at a low glucose concentration.

2 | RESULTS

2.1 | Low glucose concentration enhances HBV replication and gene expression in hepatocytes

Given glucose as one of the most important metabolic substrates in living organisms, we asked whether and how external glucose supply regulates HBV replication. Under the standard condition, HepG2.2.15 cells with stable HBV replication were grown in complete RPMI-1640 medium with 10-mM glucose. We first examined how the glucose concentration in the culture medium affected HBV replication and gene expression. HepG2.2.15 cells were cultured with different glucose concentrations (5, 10, and 25 mM). HBV RIs were prepared on Day 4 and subjected to Southern blotting analysis. The amount of HBV RIs decreased significantly with increasing glucose concentrations (Figure 1a). The levels of encapsidated and secreted HBV DNA (in HBV virions) were determined using real-time PCR. Consistently, a low glucose concentration of 5 mM in the cell culture medium led to increased levels of both intracellular and secreted HBV DNA (Figure 1a). The levels of intracellular and secreted HBsAg and HBeAg were measured by chemiluminescent microparticle immunoassay. The levels of intracellular and secreted HBsAg but not HBeAg were substantially higher under the low glucose condition (Figure 1a).

To explain the changes in HBV replication activity at different glucose concentrations, the effects of glucose concentrations on HBV gene expression were judged by determining the levels of HBV RNAs using real-time RT-PCR and the HBV promoter activity using luciferase reporter assays. The levels of HBV RNAs were highest at the glucose
concentration of 5 mM, compared with those at higher glucose concentrations (10 and 25 mM; Figure 1b). However, HBV RNA levels changed less than twofold among the different glucose concentrations in the cell cultures. The luciferase reporter assays clearly showed that single HBV promoter activity dropped slightly (Figure 1c), though the levels of some specific transcription factors, such as PGC1α, CREB,
and ChREBP, were strongly reduced at the higher glucose concentrations (Figure 1d). These results suggest that the glucose concentration modulates HBV transcription. Nevertheless, the regulation at the level of transcription was apparently not the only mechanism to determine the changed magnitude of HBV replication at different glucose concentrations.

Low glucose concentration may cause insufficient nutrition of cells. Therefore, we added pyruvate as a supplement at different glucose concentrations in the culture medium. Nevertheless, adding pyruvate did not affect HBV replication at low glucose concentration (Figure S1a, Supporting information). Next, cell proliferation was measured using a CCK8 assay at indicated time points from 6 to 72 hr after cultured in different glucose concentrations. These glucose concentrations did not affect cell proliferation of HepG2.2.15 cells at any indicated time point (Figure S1b).

Finally, PHHs were infected with HBV and cultured at glucose concentrations of 5 and 10 mM up to 10 days post infection. HBV infection in PHHs was determined by measuring levels of HBsAg and HBeAg in the culture supernatants. At low glucose concentration, the HBsAg but not HBeAg levels were markedly increased in the culture supernatants (Figure 2a), consistent with the findings in HepG2.2.15 cells. To assess the differentiation status of cultured PHHs during the course of infection and treatment, we examined the expression level of albumin by Western blotting. There was almost no change in the expression level of albumin during the experiments (Figure 2b,e), suggesting that PHHs maintained the differentiated status. In fact, HBsAg and HBeAg production usually maintained during the time frame up to 12 days (Galle et al., 1994), indicating that PHHs fully support HBV replication.

Collectively, the data suggest that low glucose concentration enhances HBV replication in hepatoma cells and PHHs. However, the changes in HBV transcription do not fully explain the difference on HBV replication activity at low and high glucose concentrations. Other cellular mechanisms may participate in the modulation of HBV replication.

2.2 | Enhanced HBV replication at low glucose concentration depends on increasing autophagic flux

AMPK is active at low glucose concentrations and interacts with ULK1, subsequently phosphorylating ULK1 at amino acid residue Ser 555, thereby initiating autophagy (Kim, Kundu, Viollet, & Guan, 2011). Furthermore, the Akt/mTOR pathway is inhibited at low glucose concentrations (Harada et al., 2009; Ryu, Lee, Yun, & Han, 2010). In addition, previous studies reported that the Akt/mTOR pathway is negatively associated with HBV replication (Bagga, Rawat, Ajenjo, & Bouchard, 2016; Lin et al., 2017; Rawat & Bouchard, 2015). Therefore, we proposed that glucose modulated HBV replication through the AMPK- and Akt/mTOR/ULK1-induced autophagy. The levels of LC3 at the indicated glucose concentrations were measured using immunofluorescence staining and Western blotting analysis. At low glucose concentration (5 mM), the numbers of endogenous LC3-positive autophagic puncta as well as HBsAg

![FIGURE 2: Glucose regulates Hepatitis B virus (HBV) infection, and the AMPK-Akt/mTOR-autophagy axis in primary human hepatocytes (PHHs).](image-url)
expression (Figure 3a) and the expression levels of LC3-II and p62 (Figure 3b) were markedly higher than those at higher glucose concentrations in HepG2.2.15 cells. Consistently, the levels of LC3-II and p62 in PHHs were higher at 5 mM glucose than at 10 mM (Figure 2b). In an additional experiment, HepG2.2.15 cells were cultured at three indicated glucose concentrations with or without chloroquine, an inhibitor of autolysosomal cargo degradation. The 5-mM glucose further permitted LC3-II accumulation (Figure S2), suggesting a stronger autophagic flux at the low glucose concentration.

To further investigate the involvement of autophagy in the modulation of HBV replication at different glucose concentrations, the autophagy-related gene ATG5 was silenced, and HBV replication, intracellular HBsAg and HBeAg levels, and HBsAg and HBeAg levels in the supernatants were measured. ATG5 silencing decreased the LC3-II levels in HepG2.2.15 cells at all three indicated glucose concentrations used for cell culture (Figure 3c). Furthermore, HBV IRs, intracellular HBV DNA levels, intracellular HBsAg levels, and HBsAg levels in the supernatants were also lower at all used glucose concentrations (Figure 3d). In summary, low glucose concentration increases autophagic flux, which is associated with enhanced HBV replication.

2.3 Glucose changes HBV replication through regulating AMPK-Akt/mTOR-dependent autophagy

Next, we examined in detail how glucose concentration modulates cellular signalling pathways and thereby regulates HBV replication. Previous studies established that autophagy is regulated by the AMPK and

![Image](https://example.com/image.png)

**FIGURE 3** Enhanced HBV replication at low glucose concentration depends on increasing autophagic flux. (a) HepG2.2.15 cells were cultured in medium with the indicated glucose concentrations (5, 10, and 25 mM) and harvested after 48 hr. The cells were fixed and incubated with a primary rabbit anti-LC3B and horse anti-HBsAg antibodies and then stained with an Alexa Fluor 488-conjugated anti-rabbit and Alexa Fluor 594-conjugated anti-horse secondary antibody IgG, respectively. The distribution of LC3 was imaged by immunofluorescence microscopy. Scale bar, 5 μm. (b) HepG2.2.15 cells were cultured in medium with the indicated glucose concentrations (5, 10, and 25 mM) and harvested after 48 hr. The LC3 and p62 expression levels were analysed by Western blotting using beta-actin as a loading control. (c) HepG2.2.15 cells were transfected with siATG5 or a control siRNA (siR-C) at 40 nM, 24 hr post transfection, the cells were cultured in medium with the indicated glucose concentrations (5, 10, and 25 mM) and harvested after 72 hr. Western blotting analysis was used to detect the ATG5 silencing effect. The LC3 and p62 expression levels were analysed by Western blotting, using beta-actin as a loading control. (d) HepG2.2.15 cells were treated as in (c). The HBsAg and HBeAg levels in the culture supernatants and intracellular HBsAg levels from cell lysates were determined as described above. The HBV DNA levels in intracellular was detected by real-time PCR. Encapsidated HBV replicative intermediates were detected by Southern blotting. *p < .05; **p < .01; ***p < .001; ns, not significant. DAPI 4',6-diamidino-2-phenylindole; HBV, Hepatitis B virus; LC3, microtubule-associated protein 1 light chain 3 beta; S/CO, signal to cutoff ratio.
mTOR signalling pathways (Kim et al., 2011; Nwadike, Williamson, Gallagher, Guan, & Chan, 2018; Xie et al., 2016; Xu et al., 2016). Therefore, we tested whether changed glucose concentrations regulated AMPK, Akt, and mTOR activities in host cells. HepG2.2.15 cells were cultured with the indicated glucose concentrations (5, 10, and 25 mM) for 48 hr. The expression of total AMPK, Akt, mTOR, and p70S6K proteins and its phosphorylated forms were detected using Western blotting. Although AMPK and ULK1 were significantly activated, Akt, mTOR, and p70S6K were inactivated at 5-mM glucose, respectively, according to the relative level of their phosphorylated glucose, respectively, due to long-term experiments, the total levels of these proteins often changed with the same tendency of the corresponding phosphorylated forms. In PHHs, 5-mM glucose in the culture medium had a similar impact on the expression levels of these proteins (Figure 2c,d). Therefore, the AMPK pathway was activated, whereas the Akt/mTOR pathway was inhibited at the low glucose concentration.

**FIGURE 4**  Low glucose concentration activates AMPK and inactivates mTOR, thereby enhancing HBV replication. (a,b) HepG2.2.15 cells were cultured in medium with the indicated glucose concentrations (5, 10, and 25 mM) and harvested after 48 hr. Western blotting analysis was performed to detect the levels of total or phosphorylated AMPK, ULK1, mTOR, AKT, and p70 S6K, using beta-actin as a loading control. (c) HepG2.2.15 cells were treated with AICAR (0.1 mM) for 72 hr. The intracellular HBsAg and HBeAg levels and the HBsAg and HBeAg levels in the culture supernatants were determined as above described. The levels of intracellular HBV DNA and that in the supernatants were determined by real-time PCR. Encapsidated HBV replicative intermediates were detected by Southern blotting. (d,e) HepG2.2.15 cells were treated with AICAR (0.1 mM) for 48 hr. Western blotting analysis was used to determine the levels of AMPK, p-AMPK, LC3, and p62 using beta-actin as a loading control. (f) PHHs were infected with HBV virions (multiplicity of infection = 30). 10 days post infection, PHHs were treated with AICAR (0.1 mM) and harvested after 48 hr. (e) The HBsAg and HBeAg levels in the culture supernatants were determined as described above. (f) Western blotting analysis was used to determine the levels of AMPK, p-AMPK, LC3, and p62 in the lysates of PHHs using beta-actin as a loading control. *p < .05; **p < .01; ***p < .001; ns, not significant. AICAR, 5-aminimidazole-4-carboxamide-1-beta-D-ribofuranoside; HBV, Hepatitis B virus; LC3, microtubule-associated protein 1 light chain 3 beta; PHH, primary human hepatocyte; RC, relaxed circular DNA; S/CO, signal to cutoff ratio; SS, single-stranded DNA.
To confirm the function of AMPK in regulation of HBV replication, the HepG2.2.15 cells were treated with an AMPK agonist AICAR for 72 hr, and the HBV DNA levels in cells and supernatants were analysed by Southern blotting hybridisation and real-time PCR, respectively. The levels of intracellular HBsAg and secreted HBsAg in the supernatant were markedly increased after AICAR treatment (Figure 4c). And the levels of intracellular and secreted HBV DNA were significantly higher in AICAR-treated cells compared with control cells regardless of the glucose concentrations (Figures 4c and S3), indicating that AMPK activity positively regulates HBV replication and virion production. Western blotting analysis showed notably increased levels of phosphorylated AMPK and ULK1 and LC3-II after AICAR treatment in HepG2.2.15 cells (Figure 4d).

In addition, PHHs were infected with HBV and treated with AICAR up to 10 days post infection. HBV infection in PHHs was determined by measuring levels of HBsAg and HBeAg in the culture supernatants. After AICAR treatment, HBsAg but not HBeAg levels were slightly increased (Figure 4e), consistent with the previous results in HepG2.2.15 cells. The levels of phosphorylated AMPK and ULK1 as well as LC3-II in PHHs were elevated after AICAR treatment (Figure 4f).

Collectively, the data suggest that AMPK positively regulated autophagy and thereby increased HBV replication in hepatoma cells and PHHs.

We also investigated the involvement of the Akt/mTOR pathway in HBV replication at different glucose concentrations. HepG2.2.15 cells were grown in the presence of Akti 1/2 or rapamycin in cultures with the indicated glucose concentrations for 72 hr. The intracellular HBV DNA levels, intracellular HBsAg and HBeAg levels, and both HBsAg and HBeAg levels in the supernatants were markedly higher in the presence of Akti 1/2 or rapamycin than in the mock control, especially if higher glucose concentrations were used (Figure 5a). The expression levels of LC3-II and p62 in HepG2.2.15 cells were higher after Akti 1/2 or rapamycin treatment (Figure 5b).

Taken together, these findings show that low glucose concentration in the medium activates the AMPK pathway but inhibits the Akt/mTOR signalling pathway to induce autophagy, thereby upregulating HBV replication.

### 2.4 GLUT inhibitor phloretin enhances HBV replication by upregulating AMPK-Akt/mTOR-induced autophagy

Glucose is uptaken by GLUTs into cells. Phloretin is a well-known inhibitor of GLUTs and reduces the speed of glucose uptake into host cells (Xintaropoulou et al., 2015). We assumed that phloretin may modulate HBV replication by mimicking the culture conditions at low glucose concentration. HepG2.2.15 cells were grown under the standard condition and treated with phloretin at 20 and 50 μM for 72 hr. Phloretin treatment at 50 μM markedly increased the levels of intracellular and secreted HBV DNA and HBsAg in the cell culture supernatants (Figure 6a). Real-time RT-PCR analysis showed that phloretin treatment also enhanced HBV RNA transcription in HepG2.2.15 cells (Figure 6b).

Next, we examined whether phloretin enhanced HBV replication by regulating AMPK and Akt/mTOR, as observed in cells cultured at the low glucose concentration. The cellular levels of total AMPK, Akt, and mTOR and their phosphorylated forms were determined using Western blotting. Phloretin treatment elevated the levels of phosphorylated AMPK and reduced Akt/mTOR phosphorylation.
(Figure 6c), proven that phloretin activated AMPK but inhibited Akt/mTOR. Furthermore, phloretin treatment increased the expression of p62 and LC3-II in HepG2.2.15 cells.

Similarly, the phloretin treatment led to slightly enhanced HBsAg production in PHHs. PHHs were infected with HBV and treated with phloretin at 20 or 50 μM up to 10 days post infection. After phloretin treatment, the HBsAg but not HBeAg levels were slightly increased in the culture supernatants (Figure 6e). Consistently, the levels of phosphorylated AMPK and ULK1 and LC3-II in PHHs were elevated after phloretin treatment, whereas decreased levels of phosphorylated Akt and mTOR were detected (Figure 6f). Thus, phloretin treatment upregulates AMPK-Akt/mTOR-induced autophagy in hepatoma cells and PHHs.

In addition, phloretin slightly enhanced glycolysis, as indicated by increased lactate production in the culture medium (Figure 6d). Finally, the presence of pyruvate did not alter the effect of phloretin on the production of HBsAg and HBeAg (Figure S4a). The proliferation of HepG2.2.15 cells was measured using the CCK8 assay, confirming that phloretin treatment did not affect cell proliferation at any indicated time point (Figure S4b).

**FIGURE 6** Phloretin enhances HBV replication and gene expression by upregulating AMPK/mTOR-ULK1-induced autophagy. (a,b) HepG2.2.15 cells were treated with 20 or 50 μM phloretin and harvested after 72 hr. (a) The HBsAg and HBeAg levels in the culture supernatants were determined as described above. The HBV DNA levels in intracellular and that in the supernatants were detected by real-time PCR. Encapsidated HBV replicative intermediates were detected by Southern blotting. (b) Real-time real-time polymerase chain reaction was performed to determine the HBV RNA levels in HepG2.2.15 cells. (c) HepG2.2.15 cells were treated with 20 or 50 μM phloretin and harvested after 48 hr. Western blotting analysis was performed to detect the levels of total or phosphorylated AMPK, Akt, mTOR, LC3, and p62 using beta-actin as a loading control. (d) HepG2.2.15 cells were treated with 20 or 50 μM phloretin and harvested after 72 hr. Lactate production was measured by a lactate colorimetric/fluorometric assay kit, according to the manufacturer's protocol. (e,f) PHHs were infected with HBV virions (multiplicity of infection = 30). Ten days post infection, PHHs were treated with phloretin (20 and 50 μM) and harvested after 48 hr. (e) The HBsAg and HBeAg levels in the culture supernatants were determined as described above. (f) Western blotting analysis was performed to detect the levels of total or phosphorylated AMPK, Akt, mTOR, LC3, and p62 using beta-actin as a loading control. *p < .05; **p < .01; ***p < .001; ns, not significant. HBV, Hepatitis B virus; LC3, microtubule-associated protein 1 light chain 3 beta; PHH, primary human hepatocyte; RC, relaxed circular DNA; S/CO, signal to cutoff ratio; SS, single-stranded DNA.
Taken these data together, phloretin treatment enhances HBV replication, upregulates AMPK/mTOR-ULK1 autophagy, and increases glycolysis in hepatoma cells.

2.5 | 2-DG decreases HBV replication and gene expression

2-DG, an analogue of glucose, is best known as an inhibitor of glycolysis and blocks the cellular HK enzymes (Brown, 1962). We addressed the question whether blocking of glycolysis affects HBV replication. Therefore, we treated the HepG2.2.15 cells with 2-DG for 72 hr. As shown in Figure 7a, 2-DG treatment significantly decreased glycolysis in HepG2.2.15 cells. Interestingly, 2-DG markedly decreased the levels of intracellular HBV RIs, extracellular HBV DNA, and intracellular HBsAg and secreted HBsAg in the supernatants in a dose-dependent manner in HepG2.2.15 cells (Figure 7b). 2-DG treatment also reduced the levels of HBV RNAs (Figure 7c). Consistent with our previous results, adding pyruvate simultaneously had no effect on the 2-DG-mediated inhibition of...
HBsAg production in HepG2.2.15 cells (Figure 7d). These data suggest that treatment with 2-DG inhibits glycolysis and HBV gene expression and replication in HepG2.2.15 cells.

2.6 2-DG treatment leads to AMPK and Akt/mTOR phosphorylation but decreased HBV replication independently on glucose concentrations

It has been reported that 2-DG treatment results in an increase in intracellular AMP/ATP ratio, thereby activating AMPK. Consistently, the level of phosphorylated AMPKα subunit (Thr172) increased, when hepatoma cells were treated with 2-DG (1, 5, and 10 mM) for 48 hr (Figure 8a). However, the levels of phosphorylated Akt (Ser473), which is required for Akt to activate its downstream targets, was enhanced, as well as mTOR phosphorylation and p70 S6K expression (Figure 8a). 2-DG also promoted the p62 and LC3-II expression in HepG2.2.15 cells, consistent with the results of previous reports (Jeon, Kim, Park, & Yun, 2015; Xi et al., 2011).

Then, we asked whether the Akt/mTOR pathway participated in the regulation of HBV replication in the presence of 2-DG. Akt and mTOR were blocked using the inhibitors Akti 1/2 and rapamycin after 2-DG treatment, respectively. Both inhibitors abrogated the suppressive effect of 2-DG on HBV replication (Figure 8c), suggesting an involvement of Akt/mTOR signalling pathway in the regulation of HBV replication in the presence of 2-DG.

**FIGURE 8** 2-DG inhibits HBV replication by upregulating the Akt/mTOR signalling pathway. (a) HepG2.2.15 cells were cultured with 2-DG (1, 5, and 10 mM) and harvested after 48 hr. The levels of total or phosphorylated AMPK, Akt, mTOR, LC3, and p62 were analysed by Western blotting using beta-actin as a loading control. (b) HepG2.2.15 cells were cultured with 2-DG (1 mM) with or without inhibitor Akti 1/2 (1 μM) or rapamycin (1 μM) for 72 hr. The HBsAg and HBeAg levels in the culture supernatants were detected as described above. Encapsidated HBV replicative intermediates were detected by Southern blotting. (c,d) HepG2.2.15 cells were cultured in medium with the indicated glucose concentrations (5, 10, and 25 mM) then treated with 1 mM 2-DG and harvested after 72 hr. (c) Encapsidated HBV replicative intermediates were detected by Southern blotting. The HBsAg and HBeAg levels in the culture supernatants were detected as described above. (d) Western blotting analysis was performed to determine the levels of Akt and p-Akt using beta-actin as a loading control. *p < .05; **p < .001; ns, not significant. 2-DG, 2-deoxy-D-glucose; HBV, Hepatitis B virus; LC3, microtubule-associated protein 1 light chain 3 beta; RC, relaxed circular DNA; S/CO, signal to cutoff ratio; SS, single-stranded DNA.
We further examined whether the suppressive effect of 2-DG on HBV was dependent on the glucose concentration in the culture medium. HepG2.2.15 cells were grown in the medium with the indicated glucose concentrations (5, 10, and 25 mM) and then treated with 2-DG (1 mM). Treatment with 2-DG markedly decreased the levels of HBV RIs and secreted HBsAg in the supernatants in HepG2.2.15 cells regardless of the glucose concentrations used (Figure 8d). The expression levels of total Akt and its phosphorylated form significantly increased in HepG2.2.15 cells regardless of the glucose concentration (Figure 8e).

In addition, 2-DG has a similar inhibitory effect on HBV infection in PHHs. PHHs were infected with HBV and treated with 2-DG (1, 5, and 10 mM) up to 10 days post infection. After 2-DG treatment, HBsAg and HBeAg levels in the culture supernatants were markedly decreased in a dose-dependent manner (Figure 9a). Consistently, the levels of phosphorylated Akt, mTOR, AMPK, and ULK1 in PHHs were increased after 2-DG treatment (Figure 9b).

The upregulated AMPK and Akt/mTOR phosphorylation by 2-DG may explain decreased HBV replication and gene expression under these culture conditions. Due to the complexity of the functions controlled by these pathways, other mechanisms may also contribute to HBV suppression and need to be considered in future study.

3 | DISCUSSION

Viruses depend on the supply of energy and building blocks for their replication (Fontaine, Sanchez, Camarda, & Lagunoff, 2015; Mahmoudabadi, Milo, & Phillips, 2017). Indeed, various metabolic pathways are essential for efficient viral replication. Here, we found that HBV replication was significantly influenced by the glucose concentration in the medium and by glucose uptake via GLUTs at the step of transcription and via AMPK-Akt/mTOR-ULK1-induced autophagy. However, its analogue 2-DG suppressed HBV replication by inhibiting the glycolytic pathway and activating the Akt/mTOR signalling pathway despite the increased AMPK and autophagic activity (Figure 10).

Defining the cellular metabolic processes related to viral infection may reveal new therapeutic targets and contribute to the development of safe and effective therapies against viral infections (Ikeda & Kato, 2007). HBV is thought to be a “metabolovirus” (Shlomai & Shaul, 2008), and its transcription is largely dependent on hepatic metabolic controls (Bar-Yishay, Shaul, & Shlomai, 2011; Tacke, Liedtke, Bocklage, Manns, & Trautwein, 2005) and cellular transcription factors (Ondracek & McLachlan, 2011; Quasdorff & Protzer, 2010; Ramiere et al., 2008). Previous studies in HBV transgenic mice have illustrated, for example, that fasting decreases glucose levels but increases HBeAg synthesis in serum. PGC1α transcripts are induced by fasting in HBV transgenic mice (L. Li, Oropeza, Kaestner, & McLachlan, 2009). In this study, the expression of transcription factors, including PGC1α, CREB, and ChREBP, was found to be altered by these indicated glucose concentrations in the cell cultures and correlated with changes in the HBV RNAs levels. This is consistent with previous studies regarding the importance of HBV transcriptional control. HBV transcriptional activity is regulated by HBV promoters and two additional enhancers and the abundance of specific hepatic transcription factors. The expression of relevant transcription factors is inversely

**FIGURE 9** 2-DG decreases Hepatitis B virus replication and gene expression by upregulating the Akt/mTOR signalling pathway in primary human hepatocytes (PHHs). Primary human hepatocytes were infected with Hepatitis B virus virions (multiplicity of infection = 30). Ten days post infection, PHHs were treated with 2-DG (1, 5, and 10 mM) and harvested after 48 hr. (a) The HBsAg and HBeAg levels in the culture supernatants were determined as described above. (b) Western blotting analysis was performed to detect the levels of total or phosphorylated AMPK, Akt, mTOR, LC3, and p62 using beta-actin as a loading control. *p < .05; **p < .01; ***p < .001; ns, not significant. 2-DG, 2-deoxy-D-glucose; LC3, microtubule-associated protein 1 light chain 3 beta; S/CO, signal to cutoff ratio
determined by glucose concentrations and correlated to the steady state levels of HBV RNAs. However, the posttranscriptional control of HBV replication at the steps of assembly, release, and degradation by autophagy has emerged as relevant and effective mechanisms that require attention in future studies on the HBV life cycle. In a number of studies, the production of HBeAg has been considered as a marker of HBV replication. However, autophagy significantly promotes HBV replication through upregulating AMPK and Akt/mTOR signalling pathway negatively regulates HBV replication (Gardner, Abcouwer, Losiewicz, & Fort, 2015; Maus et al., 2014). In this study, we found that AMPK, a key player in energy homeostasis, induces autophagy under conditions of reduced nutrient supply (Boroughs & DeBerardinis, 2015; Meijer & Codogno, 2011). AMPK stimulation is reported to inactivate mTOR by AMPK-mediated phosphorylation of both TSC2 and Raptor. ULK1 can subsequently interact with and be phosphorylated by AMPK to initiate autophagy (Corradetti, Inoki, Bardeesy, DePinho, & Guan, 2004; Gwinn et al., 2008; Kim et al., 2011; Nwadike et al., 2018). Consistent with this notion, AMPK activation occurred at low glucose concentration and in the presence of the GLUT inhibitor phloretin, along with inhibited mTOR activity, ULK1 phosphorylation, and autophagy induction. Taken together, our results reveal a novel regulatory mechanism by which glucose supply regulates HBV replication.

Glucose is an essential nutrient and energy source in living organisms. Maintaining energy homeostasis is very important in mammalian physiology. Glycolysis is considered as a “central” carbon metabolic pathway because it is the backbone of several metabolic pathways and is pivotal for energy homeostasis (Akram, 2014; Dashty, 2013; Kornberg, 2000). Herein, HBV replication decreased after 2-DG treatment in HepG2.2.15 cells. This observation highlights the notion that HBV replication requires glycolysis in host cells. Treatment with 2-DG induced AMPK and Akt/mTOR activation, two processes that may regulate HBV replication in opposite ways. Activation of the Akt/mTOR signalling pathway negatively regulates HBV replication (Guo et al., 2007; Rawat & Bouchard, 2015; Xiang & Wang, 2018) and blocks glycolysis through the regulation of GLUT transport (Altomare & Khaled, 2012), whereas AMPK acts through downstream autophagic pathway to regulate HBV replication. Treatment with 2-DG strongly inhibited HBV replication, but promoted autophagy (Figures 7–9). We assume that other pathways in the downstream of AMPK and Akt/mTOR are also involved in the regulation of HBV replication. Previously, we tested several mTOR-related pathways and found that SREBP1 was also partly activated to regulate HBV replication (Lin et al., 2017). Therefore, a detailed analysis is needed to completely dissect the functions of different pathways and their relative contributions to the control of HBV replication. On the other hand, 2-DG may markedly inhibit glycolysis that is essential for viral replication (Gardner, Abcouwer, Losiewicz, & Fort, 2015; Maus et al., 2006; Muaddi et al., 2010). Our results in this study did not support the role of reduced nutrient supply in HBV suppression as the cellular protein synthesis was not affected by 2-DG. However, these questions remain to be answered in future studies.

4 EXPERIMENTAL PROCEDURES

4.1 Cell culture and transfection

All used cell lines were cultured in a humidified atmosphere containing 5% CO₂ at 37°C. The HBV-producing HepG2.2.15 hepatoma cell line,
containing the integrated HBV genomic dimers, was routinely cultured in the standard RPMI-1640 medium with 10-mM glucose (Gibco), supplemented with 10% inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), 1% nonessential amino acids, 1% HEPES, and 500 μg/ml G418 (Merck Millipore). Different glucose concentrations were used in cell culture medium, based on glucose-free RPMI-1640 medium (11879020; Gibco), supplemented with the indicated concentrations of glucose (5, 10, and 25 mM), 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), 1% nonessential amino acids, 1% HEPES, and 500 μg/ml G418 (Merck Millipore). Primary human hepatocytes (PHHs), a gift from Dr. Ruth Broering (University Hospital Essen, Germany), was cultured in glucose-free Dulbecco’s Modified Eagle medium with the indicated concentrations of glucose (5 and 10 mM). Small interfering RNAs (siRNAs), siR-C (AllStars Negative Control siRNA, 1027280; Qiagen), and siATG5 (hs_APG5L_6 FlexiTube siRNA, S102655310; Qiagen) were transfected into cells at 40 nM using Lipofectamine 2000 transfection reagent (Invitrogen).

4.2 Chemical reagents

Glucose (G8270), 2-DG (D8375), phloretin (P7912), Akt1/2 (124018), rapamycin (R8781), and chloroquine (C6628) were purchased from Sigma-Aldrich. AlICAR (S1802) was purchased from Selleck Chemicals.

4.3 Detection of HBV gene expression and replication

The method for the detection of HBV progeny DNA in the culture supernatants has been described previously (Lin et al., 2017; Zhang et al., 2011). HBV RNA levels in cells were measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays (Qiagen, 204154) using the primers 5’-CAGGTGCGTCTTATCTCTT-3’ (forward) and 5’-TATCTTCCCTCCCCCACTCC-3’ (reverse). The mRNA levels were normalized to the beta-actin mRNA level. These primers cover all four HBV RNAs as described previously (Zhang et al., 2011). The detection of intracellular HBsAg and HBeAg levels from cell lysates and both in the culture supernatants was performed by chemiluminescent microparticle immunoassay (; Abbott Laboratories, Chicago, IL, USA). HBV replicative intermediates (RIIs) from intracellular core particles were extracted and detected by Southern blotting as described previously (Lin et al., 2017; J. Wu et al., 2007; Zhang et al., 2011). To extract HBV RIIs from intracellular core particles, cells were lysed with lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM MgCl2, 1% NP-40) for 10 min. The cytoplasmic fraction was separated from the nuclear fraction by centrifugation. The supernatants were taken to the new Eppendorf tubes, mixed with 10 mM MgCl2 and 500 μg/ml DNasel (Roche, Germany), and incubated for 1 hr at 37°C. The reaction was terminated using 25 mM EDTA. Sodium dodecyl sulfate and proteinase K (QIAGEN) were added separately and incubated for 2 hr at 56°C. Thereafter, the DNA was extracted with phenol/chloroform (1:1) and precipitated with isopropanol. The precipitated nucleic acid was resuspended in 15-μl TE buffer. The preparation containing HBV DNA was then subjected to electrophoresis on a 0.8% agarose gel, followed by blotting onto a Hybond-N+ membrane. Blots were hybridised with 32P-labelled HBV DNA probes, which were prepared using a random priming labelling kit (GE Healthcare, RPN1633) in hybridization buffer (G-Biosciences, 786-160). The signals were visualised and analysed using a phosphomagen (Cyclon, Packard Instrument).

4.4 Western blotting assays

The methods for preparing whole cell protein lysates and Western blotting have been described previously (Lin et al., 2017). Antibodies against the following proteins were used: anti-PGC1α (2178S), anti-CREB (9197S), anti-AMPK (2532S), anti-phospho-AMPK (2531S), anti-Akt (92725), anti-phospho-Akt (Ser473; 92715), anti-mTOR (29725), anti-phospho-mTOR (29715), anti-p70 S6K (92025), anti-phospho-p70 S6K (S371; 92345), anti-ULK1 (4773S), anti-phospho-ULK1 (Ser555; 58695), anti-p62 (51145), anti-LC3B (38685), anti-ChREBP (abcam, ab112944), anti-Albumin (49295), and anti-beta-actin (Sigma, A5441).

4.5 Immunofluorescence staining

Immunofluorescence staining was performed as described previously (Lin et al., 2017). Briefly, HepG2.2.15 cells were grown on coverslips and treated as indicated in each experiment. Then, the cells were fixed in 4% paraformaldehyde and permeabilised with 0.1% Triton X-100 and incubated with anti-LC3B antibodies, staining with Alexa Fluo 488-conjugated Goat anti-Rabbit IgG (H + L). The distribution of LC3B protein was observed with a Zeiss ELYRA PS.1 SIM/PAL. Cell proliferation was determined by a cell counting Kit-8 assay kit (Sigma-Aldrich, 96992) according to the manufacturer’s protocol.

4.6 Luciferase reporter assay

The detection of firefly luciferase activity was performed by the DualGlo luciferase reporter assay system (Promega, E2940) and normalized to the internal control Renilla luciferase activity. Four luciferase reporter plasmids, pSP1 (nt 2224-1853), pXP (nt 1237-1375), based on pGL3 basic were constructed with HBV promoters previously and used in the assay as described (Zhang et al., 2011).

4.7 Cell proliferation assay

Cell proliferation was determined by a cell counting Kit-8 assay kit (Sigma-Aldrich, 96992) according to the manufacturer’s protocol.
4.8 Lactate assay

Accumulation of lactate in the culture medium was measured by a lactate colorimetric/fluorometric assay kit (BioVision Inc, K607) according to the manufacturer’s protocol.

4.9 Statistical analyses

Data are shown in mean ± standard error of the mean. Statistical analyses were performed using Graph Pad Prism software version 7 (La Jolla, CA, USA). ANOVA with two-tailed Student’s t test or by one-way ANOVA with a Tukey posttest was used to determine significant differences. Differences were considered statistically significant when p < .05. All experiments were repeated independently at least three times.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (TRR60) and a scholarship from the Medical Faculty of University Duisburg-Essen. The authors declare no conflicts of interest. We thank Elsevier for English language editing.

ORCID

Mengji Lu https://orcid.org/0000-0003-4287-9941

REFERENCES

Abrantes, J. L., Alves, C. M., Costa, J., Almeida, F. C., Sola-Penna, M., Fontes, C. F., & Souza, T. M. (2012). Herpes simplex type 1 activates glycolysis through engagement of the enzyme 6-phosphofructo-1-kinase (PFK-1). Biochimica et Biophysica Acta, 1822(8), 1198–1206. https://doi.org/10.1016/j.bbadis.2012.04.011

Adeva-Andany, M. M., Perez-Felpete, N., Fernandez-Fernandez, C., Donapetry-Garcia, C., & Pazos-Garcia, C. (2016). Liver glucose metabolism in humans. Bioscience Reports, 36(6), e00416. https://doi.org/10.1042/BSR20160385

Akram, M. (2014). Citric acid cycle and role of its intermediates in metabolism. Cell Biochemistry and Biophysics, 68(3), 475–478. https://doi.org/10.1007/s12013-013-9750-1

Altomare, D. A., & Khaled, A. R. (2012). Homeostasis and the importance for a balance between AKT/mTOR activity and intracellular signaling. Current Medicinal Chemistry, 19(22), 3748–3762. https://doi.org/10.2174/092986712801661130

Bagga, S., Rawat, S., Ajenjo, M., & Bouchard, M. J. (2016). Hepatitis B virus (HBV) X protein-mediated regulation of hepatocyte metabolic pathways affects viral replication. Virology, 498, 9–22. https://doi.org/10.1016/j.virol.2016.08.006

Bar-Yishay, I., Shaul, Y., & Shlomai, A. (2011). Hepatocyte metabolic signaling pathways and regulation of hepatitis B virus expression. Liver International, 31(3), 282–290. https://doi.org/10.1111/j.1478-3231.2010.02423.x

Boroughs, L. K., & DeBerardinis, R. J. (2015). Metabolic pathways promoting cancer cell survival and growth. Nature Cell Biology, 17(4), 351–359. https://doi.org/10.1038/ncb3124

Brown, J. (1962). Effects of 2-deoxyglucose on carbohydrate metabolism: Review of the literature and studies in the rat. Metabolism, 11, 1098–1112.

Chen, G., Liang, G., Ou, J., Goldstein, J. L., & Brown, M. S. (2004). Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proceedings of the National Academy of Sciences of the United States of America, 101(31), 11245–11250. https://doi.org/10.1073/pnas.0404297101

Corradetti, M. N., Inoki, K., Bardeesy, N., DePinho, R. A., & Guan, K. L. (2004). Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes & Development, 18(13), 1533–1538. https://doi.org/10.1101/gad.1199104

Dashy, M. (2013). A quick look at biochemistry: Carbohydrate metabolism. Clinical Biochemistry, 46(15), 1339–1352. https://doi.org/10.1016/j.clinbiochem.2013.04.027

DeBerardinis, R. J., & Chandel, N. S. (2016). Fundamentals of cancer metabolism. Science Advances, 2(5), e1600200. https://doi.org/10.1126/sciadv.1600200

Fontaine, K. A., Sanchez, E. L., Camarda, R., & Lagunoff, M. (2015). Dengue virus induces and requires glycolysis for optimal replication. Journal of Virology, 89(4), 2358–2366. https://doi.org/10.1128/JVI.02309-14

Galle, P. R., Hagelstein, J., Kommerell, B., Volkman, M., Scharzen, P., & Zentgraf, H. (1994). In vitro experimental infection of primary human hepatocytes with hepatitis B virus. Gastroenterology, 106(3), 664–673. https://doi.org/10.1016/0016-5085(94)90700-5

Ganem, D., & Prince, A. M. (2004). Hepatitis B virus infection—natural history and clinical consequences. The New England Journal of Medicine, 350(11), 1118–1129. https://doi.org/10.1056/NEJMra031087

Gardner, T. W., Abcouwer, S. F., Losiewicz, M. K., & Fort, P. E. (2015). Phosphatase control of 4E-BP1 phosphorylation state is central for glycolytic regulation of retinal protein synthesis. American Journal of Physiology. Endocrinology and Metabolism, 309(6), E546–E556. https://doi.org/10.1152/ajpendo.00180.2015

Guo, H., Zhou, T., Jiang, D., Cucunatì, A., Xiao, G. H., Block, T. M., & Guo, J. T. (2007). Regulation of hepatitis B virus replication by the phosphatidylinositol 3-kinase-akt signal transduction pathway. Journal of Virology, 81(18), 10072–10080. https://doi.org/10.1128/JVI.00541-07

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S.,... Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular Cell, 30(2), 214–226. https://doi.org/10.1016/j.molcel.2008.03.003

Harada, H., Itasaka, S., Kizaka-Kondoh, S., Shibuya, K., Morinibu, A., Shinomiya, K., & Hiraoka, M. (2009). The Akt/mTOR pathway assures autophagy through direct phosphorylation of Ulk1. Proceedings of the National Academy of Sciences, 106(23), 9750–9755. https://doi.org/10.1073/pnas.0901821106

Hilt, P., Schieber, A., Yildirim, C., Arnold, G., Kläber, I., Conrad, J.,... Carle, R. (2003). Detection of photolysis in strawberries (Fragaria x ananassa Duch) by HPLC-PAH-MS/MS and NMR spectroscopy. Journal of Agricultural and Food Chemistry, 51(10), 2896–2899. https://doi.org/10.1021/jf021115k

Ikeda, M., & Kato, N. (2007). Modulation of host metabolism as a target of new antivirals. Advanced Drug Delivery Reviews, 59(12), 1277–1289. https://doi.org/10.1016/j.addr.2007.03.021

Jeon, J. Y., Kim, S. W., Park, C. K., & Yun, M. (2015). The bifunctional autophagic flux by 2-deoxyglucose to control survival or growth of prostate cancer cells. BMC Cancer, 15, 623. https://doi.org/10.1186/s12885-015-1640-z

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature Cell Biology, 13(2), 132–141. https://doi.org/10.1038/ncb2152
of miR-192-3p-XIAP through NF kappa B signaling. Hepatology, 69(3), 974–992. https://doi.org/10.1002/hep.30248

Wang, M. D., Wu, H., Huang, S., Zhang, H. L., Qin, C. J., Zhao, L. H., ... Wang, H. Y. (2016). HBx regulates fatty acid oxidation to promote hepatocellular carcinoma survival during metabolic stress. Oncotarget, 7(6), 6711–6726. https://doi.org/10.18632/oncotarget.6817

Wu, J., Lu, M., Meng, Z., Trippler, M., Broering, R., Szczeponek, A., ... Schlaak, J. F. (2007). Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. Hepatology, 46(6), 1769–1778. https://doi.org/10.1002/hep.21897

Wu, Y. L., Peng, X. E., Zhu, Y. B., Yan, X. L., Chen, W. N., & Lin, X. (2016). Hepatitis B virus x protein induces hepatic steatosis by enhancing the expression of liver fatty acid binding protein. Journal of Virology, 90(4), 1729–1740. https://doi.org/10.1128/JVI.02604-15

Xi, H., Kurtoglu, M., Liu, H., Wangpaichitr, M., You, M., Liu, X., ... Lampidis, T. J. (2011). 2-Deoxy-D-glucose activates autophagy via endoplasmic reticulum stress rather than ATP depletion. Cancer Chemotherapy and Pharmacology, 67(4), 899–910. https://doi.org/10.1007/s00280-010-1391-0

Xiang, K., & Wang, B. (2018). Role of the PI3KAKTmTOR pathway in hepatitis B virus infection and replication. Molecular Medicine Reports, 17(3), 4713–4719. https://doi.org/10.3892/mmr.2018.8395

Xie, N., Yuan, K., Zhou, L., Wang, K., Chen, H. N., Lei, Y., ... Huang, C. (2016). PRKAA/AMPK restricts HBV replication through promotion of autophagic degradation. Autophagy, 12(9), 1507–1520. https://doi.org/10.1080/15548627.2016.1191857

Xintaropoulou, C., Ward, C., Wise, A., Marston, H., Turnbull, A., & Langdon, S. P. (2015). A comparative analysis of inhibitors of the glycolysis pathway in breast and ovarian cancer cell line models. Oncotarget, 6(28), 25677–25695. https://doi.org/10.18632/oncotarget.4499

Yang, F., Yan, S., He, Y., Wang, F., Song, S., Guo, Y., ... Sun, S. (2008). Expression of hepatitis B virus proteins in transgenic mice alters lipid metabolism and induces oxidative stress in the liver. Journal of Hepatology, 48(1), 12–19. https://doi.org/10.1016/j.jhep.2007.06.021

Zhang, X., Zhang, E., Ma, Z., Pei, R., Jiang, M., Schlaak, J. F., ... Lu, M. (2011). Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology, 53(5), 1476–1485. https://doi.org/10.1002/hep.24195

How to cite this article: Wang X, Lin Y, Kemper T, et al. AMPK and Akt/mTOR signalling pathways participate in glucose-mediated regulation of hepatitis B virus replication and cellular autophagy. Cellular Microbiology. 2020;22:e13131. https://doi.org/10.1111/cmi.13131

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.