Redox Biology 50 (2022) 102246

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Aquaporin-9 facilitates liver regeneration following hepatectomy

Bo Zhang [a,1], Dongyue Lv [a,1], Yang Chen [a], Weijue Nie [a], Yang Jiao [a], Junqi Zhang [a], Xiaoxiao Zhou [a], Xiao Wu [a], Siqing Chen [b], Tonghui Ma [a,*]

[a] School of Medicine & Holistic Integrative Medicine, Nanjing University of Chinese Medicine, Nanjing, PR China
[b] Jiangsu Provincial Hospital of Chinese Medicine, Nanjing, PR China

ARTICLE INFO

Keywords:
Liver regeneration
Aquaporin-9
Partial hepatectomy
Hydrogen peroxide
Oxidative stress
Gluconeogenesis

ABSTRACT

Aquaporin-9 (AQP9) is an aquaglyceroporin strongly expressed in the basolateral membrane of hepatocytes facing the sinusoids. AQP9 is permeable to hydrogen peroxide (H_2O_2) and glycerol as well as to water. Here, we report impaired liver regeneration in AQP9$^{-/-}$ mice which involves altered steady-state H_2O_2 concentration and glucose metabolism in hepatocytes. AQP9$^{-/-}$ mice showed remarkably delayed liver regeneration and increased mortality following 70% or 90% partial hepatectomy. Compared to AQP9$^{+/+}$ littermates, AQP9$^{-/-}$ mice showed significantly greater hepatic H_2O_2 concentration and more severe liver injury. Fluorescence measurements indicated impaired H_2O_2 transport across plasma membrane of primary cultured hepatocytes from AQP9$^{-/-}$ mice, supporting the hypothesis that AQP9 deficiency results in H_2O_2 accumulation and oxidative injury in regenerating liver because of reduced export of intracellular H_2O_2 from hepatocytes. The H_2O_2 overload in AQP9$^{-/-}$ hepatocytes reduced PI3K-Akt and insulin signaling, inhibited autophagy and promoted apoptosis, resulting in impaired proliferation and increased cell death. In addition, hepatocytes from AQP9$^{-/-}$ mice had low liver glycerol and high blood glycerol levels, suggesting decreased glycerol uptake and gluconeogenesis in AQP9$^{-/-}$ hepatocytes. Adeno-associated virus (AAV)-mediated expression of hepatic expression of aquaglyceroporins AQP9 and AQP3 in AQP9$^{-/-}$ mice, but not water-selective channel AQP4, fully rescued the impaired liver regeneration phenotype as well as the oxidative injury and abnormal glucose metabolism. Our data revealed a pivotal role of AQP9 in liver regeneration by regulating hepatocyte H_2O_2 homeostasis and glucose metabolism, suggesting AQP9 as a novel target to enhance liver regeneration following injury, surgical resection or transplantation.

1. Introduction

Liver regeneration is a complex and sophisticated tissue regeneration process that involves a variety of cytokines, growth factors, and metabolic signals [1,2]. The current understanding of liver regeneration mainly comes from partial hepatectomy (PH) models [3]. After PH, the remaining hepatocytes re-enter the cell cycle from a quiescent, highly differentiated state, and proliferate rapidly to compensate for the resected tissue [4]. During rapid liver regeneration hydrogen peroxide (H_2O_2) is produced in response to stimulation by growth factors, chemokines and physical stressors [5–8]. Under physiological conditions H_2O_2 flux across the hepatocyte plasma membrane contributes to cell proliferation, differentiation and angiogenesis [9,10]. However, super-physiological concentrations of H_2O_2 can cause oxidative stress, leading to hepatocyte growth arrest, cell death and tissue pathology [6,11].

Some isoforms of the aquaporin (AQP) superfamily have been reported to facilitate H_2O_2 diffusion across biological membranes [12]. These H_2O_2-permeable AQPs, now called peroxiporins, are proposed as key players to regulate redox signaling in various organisms [9,12]. In mammals, AQP9 is an aquaglyceroporin localized in the plasma membrane of hepatocytes facing the sinusoids [13,14] that transports H_2O, glycerol and some other neutral solutes [15]. AQP9 is also a peroxiporin that efficiently transports H_2O_2 [16].

Following PH there are marked changes in metabolism to adapt to the stress [17]. In mice, blood glucose is greatly reduced within 12 h after PH, body fat is rapidly mobilized, and catabolism is increased [4,18]. During this process the liver undergoes a series of adaptive changes including enhanced gluconeogenesis and reduced glycolysis to restore blood glucose homeostasis [19]. Experimental animal studies have

* Corresponding author.
E-mail address: matonghui@njucm.edu.cn (T. Ma).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.redox.2022.102246
Received 15 December 2021; Received in revised form 6 January 2022; Accepted 19 January 2022
Available online 22 January 2022

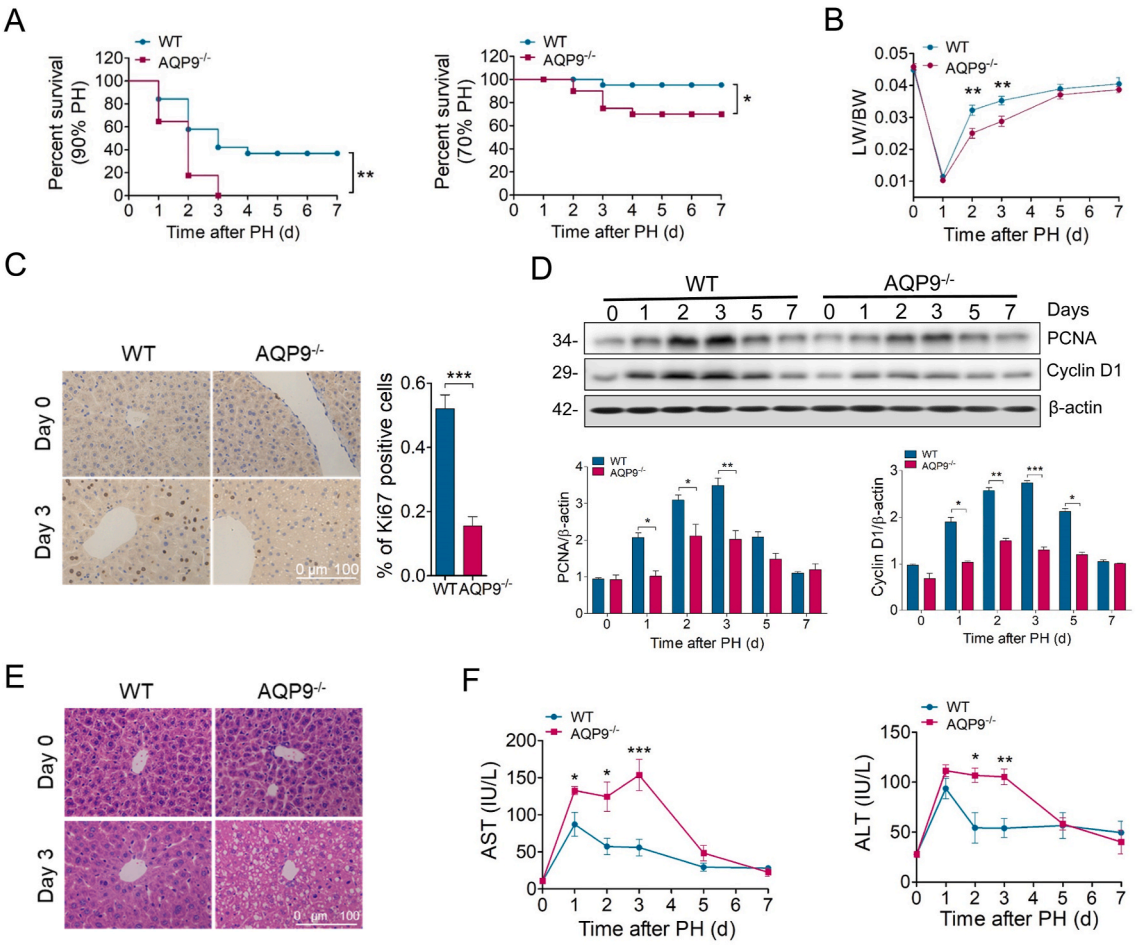

Fig. 1. *AQP9-deficiency retarded liver mass recovery and aggravate liver injury in mice after PH.* (A) Survival of AQP9$^{-/-}$ and AQP9$^{+/+}$ mice (n = 18/group) after 90% PH (*Left*) and 70% PH (*Right*). (B) Liver to body weight ratio of AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated time points after 70% PH (n = 12/group). (C) *Left*: Ki-67 immunostaining of liver tissues in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at day 0 and day 3 after 70% PH (scale bar = 100 μm); *Right:* Ki-67-positive cell counts in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at day 3 after PH (n = 5/group). (D) Western blot analysis of PCNA and Cyclin D1 protein levels in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated time points after 70% PH (n = 6/group). The number on the left of the blot represents the molecular weight of the target protein. (E) H&E staining of liver tissues in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at day 0 and day 3 after 70% PH (scale bar = 100 μm). (F) The activity of AST and ALT was measured in serum of AQP9$^{-/-}$ mice and AQP9$^{+/+}$ mice at indicated time points after 70% PH (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are expressed as mean ± S.E.M.

indicated that the altered glucose homeostasis during liver regeneration can lead to aplastic disorders and even death [20].

Glycerol is an important substrate of gluconeogenesis in liver. Hepatic glycerol mainly comes from blood glycerol released by adipose tissue through enhanced lipolysis [21,22]. Adipocytes release glycerol through aquaglyceroporin AQP7 in the plasma membrane during lipolysis and hepatocytes take up glycerol through AQP9 from sinusoidal blood [23]. Glycerol entering hepatocytes is converted to glycerol-3-phosphate by glycerol kinase, which can be used as substrate for gluconeogenesis or ATP production through glycerol-3-phosphate shuttling [21,24]. Although some studies have suggested functional involvement of AQP9 in liver glycerol transport and metabolic disorders [25–28], the impact of AQP9 on glucose and lipid metabolism during liver regeneration remains unknown.

In the present study, we demonstrate an important role of AQP9 in liver regeneration that involves modulation of oxidative stress and glucose metabolism through its H_2O_2 and glycerol transporting functions.

2. Materials and Methods

2.1. Animal procedures

All animal experiments in this study were approved by the Nanjing University of Chinese medicine and were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). AQP9 knockout mouse model in C57BL/6J background were generated by contract in the Model Animal Research Center of Nanjing University. AQP9$^{+/-}$ mice were mated to generate litter-matched AQP9$^{-/-}$ and AQP9$^{+/+}$ mice in the specific pathogen-free (SPF) facility of Nanjing University of Chinese Medicine. All mice were maintained on regular chow diet and water ad libitum (unless specified) with a 12 h light/12 h dark schedule (9 a.m.–9 p.m. light). Blood and tissue samples during liver regeneration were collected under isoflurane anesthesia for biochemical analysis, liver/body weight ratio calculation, histological analysis and immunostaining. Please see Supplemental Methods for other parts of Materials and Methods.

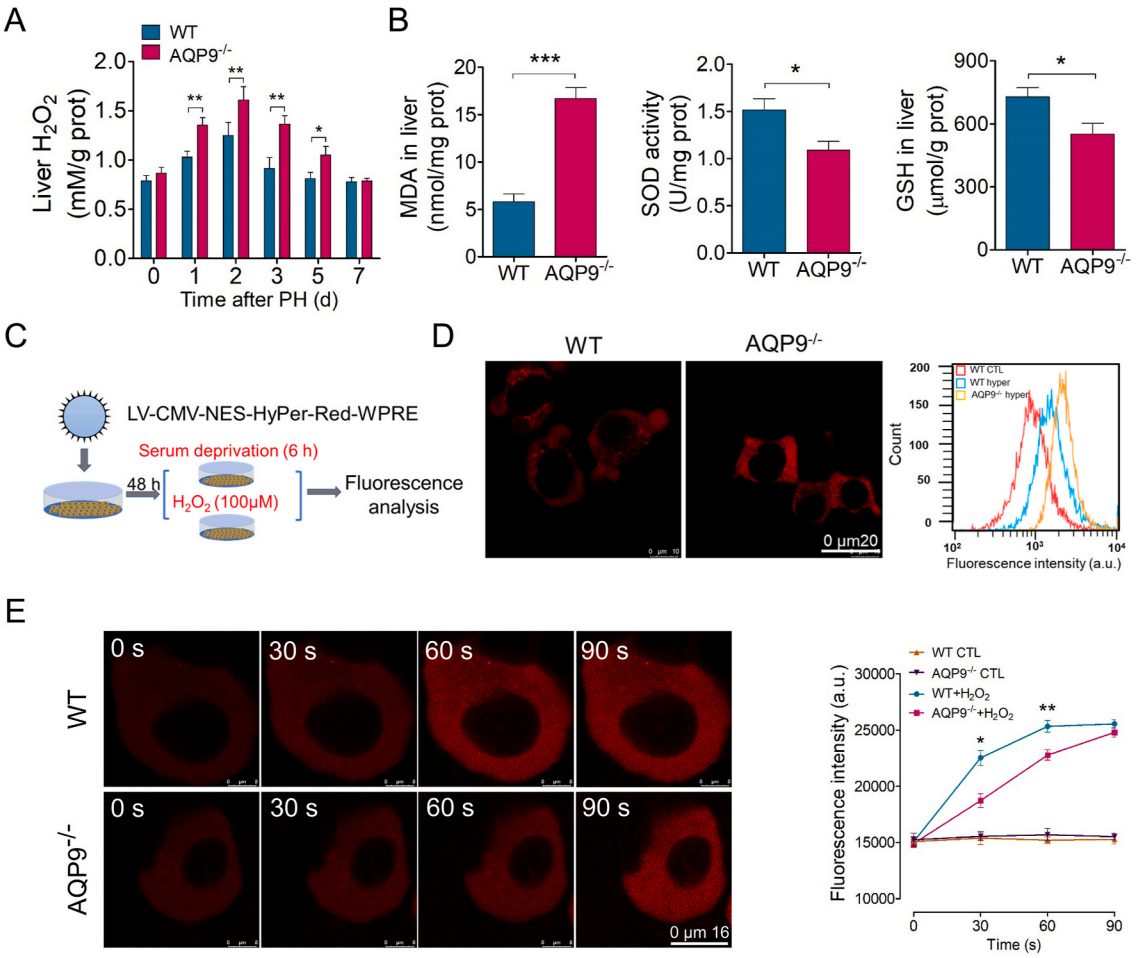

Fig. 2. *AQP9-deficiency leads to increased H_2O_2 level and redox imbalance in hepatocytes.* (A) H_2O_2 in $AQP9^{-/-}$ and $AQP9^{+/+}$ mouse liver at indicated time points after 70% PH (n = 6/group). (B) Alteration of GSH, SOD and MDA in $AQP9^{-/-}$ and $AQP9^{+/+}$ liver at day 3 after 70% PH (n = 6/group). (C) Schematic diagram of lentivirus transfection of primary hepatocytes. (D) *Left:* Representative fluorescent images of HyperRed transduced primary hepatocytes isolated from $AQP9^{-/-}$ and $AQP9^{+/+}$ mouse liver (scale bar = 100 μm). *Right:* FACS analysis of H_2O_2-dependent fluorescence in primary hepatocytes expressing HyperRed. Fluorescence was activated at 575 nm. (E) *Left:* Representative fluorescent images of HyperRed transduced primary hepatocytes incubated with 100 μM H_2O_2 (scale bar = 100 μm). *Right:* The fluorescence intensity analyzed by fluorimeter. A lentivirus without HyperRed was transfected into primary hepatocytes as control groups. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are expressed as mean ± S.E.M.

3. Results and discussion

3.1. Detained liver regeneration in $AQP9^{-/-}$ mice after PH

To investigate the role of AQP9 in liver regeneration, we performed 70% and 90% partial hepatectomy (PH) on litter-matched $AQP9^{-/-}$ and $AQP9^{+/+}$ mice. As shown in Fig. 1A, in both cases the survival rate of $AQP9^{-/-}$ mice was significantly lower than that of $AQP9^{+/+}$ group. Since $AQP9^{-/-}$ mice could not survive over 3 days after 90% PH, subsequent experiments were conducted with 70% PH. The liver-to-body weight ratio of $AQP9^{-/-}$ mice was significantly lower than $AQP9^{+/+}$ group at days 2 and 3 after 70% PH (Fig. 1B).

There were remarkably fewer Ki67-positive hepatocytes in $AQP9^{-/-}$ liver at 3 days after 70% PH (Fig. 1C). Immunoblot showed significantly lower cyclin D1 and PCNA in $AQP9^{-/-}$ livers after PH (Fig. 1D). HE staining of liver paraffin sections showed much greater vacuolar degeneration in $AQP9^{-/-}$ than $AQP9^{+/+}$ mice (Fig. 1E). Serum AST and ALT levels were significantly higher at days 2–4 after PH in $AQP9^{-/-}$ mice (Fig. 1F). These results indicate impaired hepatocyte proliferation and liver regeneration with consequently more severe hepatic injury in $AQP9^{-/-}$ mice after PH.

3.2. Higher H_2O_2 content and redox imbalance in $AQP9^{-/-}$ regenerating liver

Growth factors induce the generation of H_2O_2 as an important part of their signal transduction mechanism [29–32]. However, H_2O_2 overproduction in hepatocytes during the proliferative phase of liver regeneration can cause oxidative stress and hepatocytes death if not properly modulated [6,7,33]. Up-regulation of AQP9 was reported in PH-induced liver regeneration [34]. Therefore, AQP9 may have a key role in maintaining H_2O_2 homeostasis and reducing oxidative injury in hepatocytes in regenerating liver.

Experiments were done to investigate the role of AQP9 in hepatocyte H_2O_2 transport and oxidative injury in regenerating liver. Biochemical analysis indicated significantly higher H_2O_2 levels in $AQP9^{-/-}$ liver in the proliferative phase of regeneration after PH (Fig. 2A). The MDA level was remarkably higher and SOD and GSH levels lower in $AQP9^{-/-}$ vs. $AQP9^{+/+}$ regenerating liver (Fig. 2B). H_2O_2 in hepatocytes was measured by live cell imaging with a lentivirus-transduced H_2O_2-sensitive fluorescent protein HyPerRed (Fig. 2C). In primary cultured hepatocytes induced to produce endogenous H_2O_2 by serum deprivation, HyPerRed imaging and fluorescence-activated cell sorting indicated higher H_2O_2 level in the cytoplasm of $AQP9^{-/-}$ vs. $AQP9^{+/+}$ hepatocytes (Fig. 2D), and uptake of exogenous H_2O_2 was significantly lower in

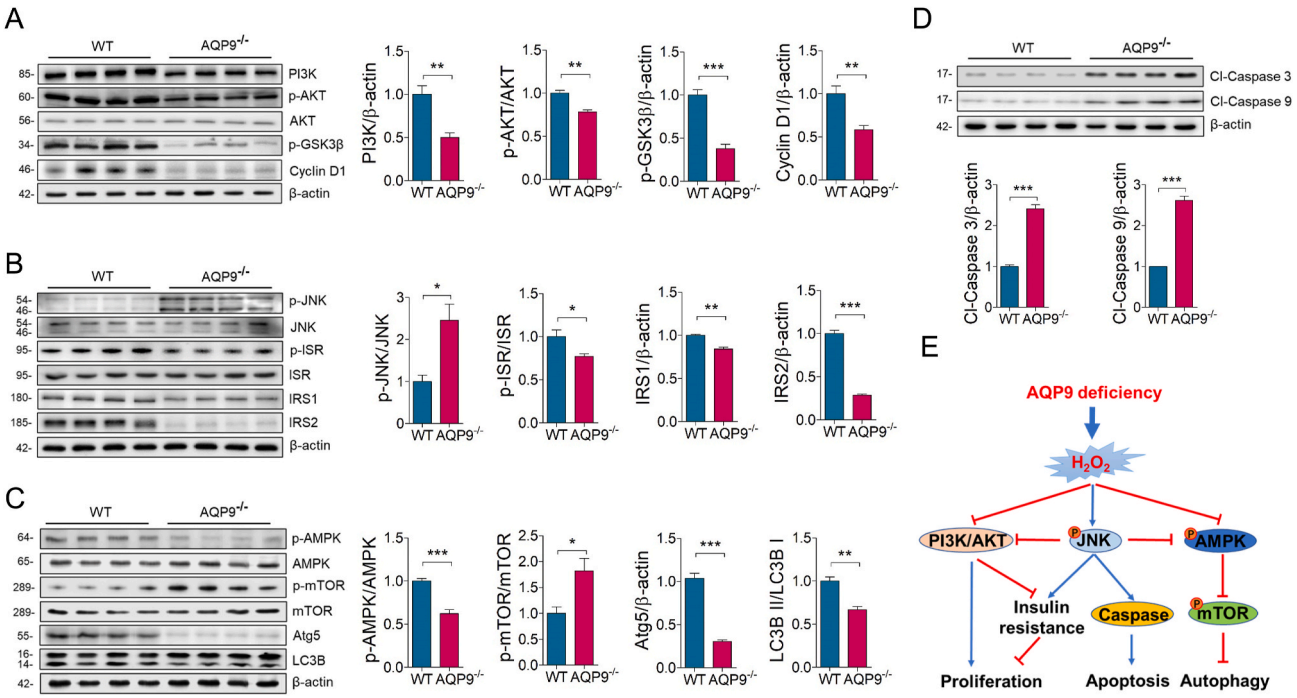

Fig. 3. *AQP9-deficiency results in inhibited PI3K-Akt and insulin signaling, enhanced apoptosis and reduced autophagy in hepatocytes.* Western blot analysis was performed using primary hepatocytes isolated from AQP9$^{-/-}$ and AQP9$^{+/+}$ liver at day 3 after 70% PH (n = 4/group). (A) PI3K-Akt signaling, including PI3K, *p*-AKT, AKT and downstream *p*-GSK3β and Cyclin D1 proteins. (B) Insulin receptor signaling, including *p*-ISR, ISR, IRS1 and IRS2 and *p*-JNK, JNK proteins. (C) AMPK-mTOR signaling, including *p*-AMPK, AMPK, *p*-mTOR, mTOR and downstream autophagic proteins Atg5 and LC3B. (D) Apoptosis: Cl-caspase 3 and Cl-caspase 9 protein levels. The number on the left of the blot represents the molecular weight of the target protein. (E) Schematic of signaling pathway changes caused by elevated H_2O_2 in hepatocytes after PH due to AQP9 deficiency. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are expressed as mean ± S.E.M.

AQP9$^{-/-}$ vs. AQP9$^{+/+}$ hepatocytes (Fig. 2E). These results support a key role of AQP9 in hepatocyte H_2O_2 transport. AQP9 deficiency reduced export of H_2O_2, increased oxidative stress and decreased antioxidant capacity in hepatocytes, which can lead to more severe liver injury during regeneration.

3.3. AQP9-deficiency impaired hepatocyte PI3K-Akt and insulin signaling, reduced autophagy and enhanced apoptosis

To elucidate the mechanisms of the impaired liver regeneration due to abnormal H_2O_2 accumulation in AQP9$^{-/-}$ hepatocytes, we analyzed several major signaling pathways that may be involved. PI3K, *p*-AKT and downstream *p*-GSK3β in the PI3K-AKT signaling pathway (Fig. 3A) and *p*-ISR, IRS1/2 in the insulin signaling pathway (Fig. 3B) were remarkably decreased in AQP9$^{-/-}$ *vs.* AQP9$^{+/+}$ hepatocytes, whereas the STAT3 signaling pathway was not changed (Supplement Fig. 1A). Analysis of autophagy-associated proteins revealed significantly decreased *p*-AMPK, increased *p*-mTOR, and lower downstream Atg5 and LC3B in AQP9$^{-/-}$ hepatocytes (Fig. 3C). Enhanced apoptosis was observed by increased cleaved caspase 3 and caspase 9 in AQP9$^{-/-}$ hepatocytes (Fig. 3D). Ferroptosis was not different as indicated by similar GPX4 and p53 (Supplement Fig. 1B). There were no significant differences in representative proteins involved in regulating the initiation (TNF-α) and termination (TGF-β) of liver regeneration (Supplement Fig. 1C). These results demonstrate that AQP9 deficiency induced apoptosis, and reduced proliferation and protective autophagy of hepatocytes (summarized in Fig. 3E), but did not affect the initiation and termination phases of liver regeneration.

ROS-mediated insulin resistance was reported to impair liver regeneration, which involved JNK activation and inhibited ISR-IRS signaling which results in delayed proliferation and increased death of hepatocytes after PH [35]. Here, we found remarkably increased phosphorylation of JNK (Fig. 3B) and decreased *p*-ISR and IRS1/2, indicating insulin resistance induced by H_2O_2 overload in AQP9$^{-/-}$ hepatocytes in regenerating liver.

3.4. Defective liver glucose metabolism in AQP9$^{-/-}$ mice

AQP9 is the major hepatocyte aquaglyceroporin that mediates the entry of glycerol from blood for gluconeogenesis [22]. We analyzed glycerol content in mouse liver and serum after PH. As shown in Fig. 4A, glycerol levels in AQP9$^{-/-}$ liver were significantly lower than AQP9$^{+/+}$ mice in regenerating liver. Corresponding serum glycerol levels were higher in AQP9$^{-/-}$ mice (Fig. 4B). Also, serum glucose levels were significantly lower in AQP9$^{-/-}$ *vs.* AQP9$^{+/+}$ mice (Fig. 4C). PAS staining and fluorescence of AQP9-tdTomato on the same cryosection indicated an overlapping distribution of glycogen and AQP9 expression (Fig. 4D). PAS staining and biochemical analysis showed much lower glycogen content in AQP9$^{-/-}$ livers at days 1–3 after PH (Fig. 4E). Immunoblot indicated significantly lower glycerol kinase (GK) and glycerol-3-phosphate dehydrogenase 1 (GPD1), and greater phosphorylated glycogen synthase (p-GS) and liver glycogen phosphorylase (PYGL), in AQP9$^{-/-}$ hepatocytes (Fig. 4F). These results indicate impaired glycerol uptake of hepatocytes in AQP9$^{-/-}$ mice, resulting in decreased glycerol gluconeogenesis and glycogen synthesis and enhanced glycogenolysis in regenerating liver.

Glycerol is an important metabolite for the control of glucose homeostasis [21]. AQP7 and AQP9 are the glycerol channels in adipocytes and hepatocytes, respectively. The coordinated regulation of the two channels leads to the optimum balance between release of glycerol through AQP7 by adipose tissue and its uptake through AQP9 by the liver [22,36,37]. Up-regulation of adipose AQP7 and down-regulation of hepatic AQP9 was connected with insulin resistance [38]. Therefore, the insulin resistance identified in AQP9$^{-/-}$ hepatocytes may involve both oxidative stress and metabolic disorders due to impaired export of H_2O_2 and insufficient uptake of glycerol.

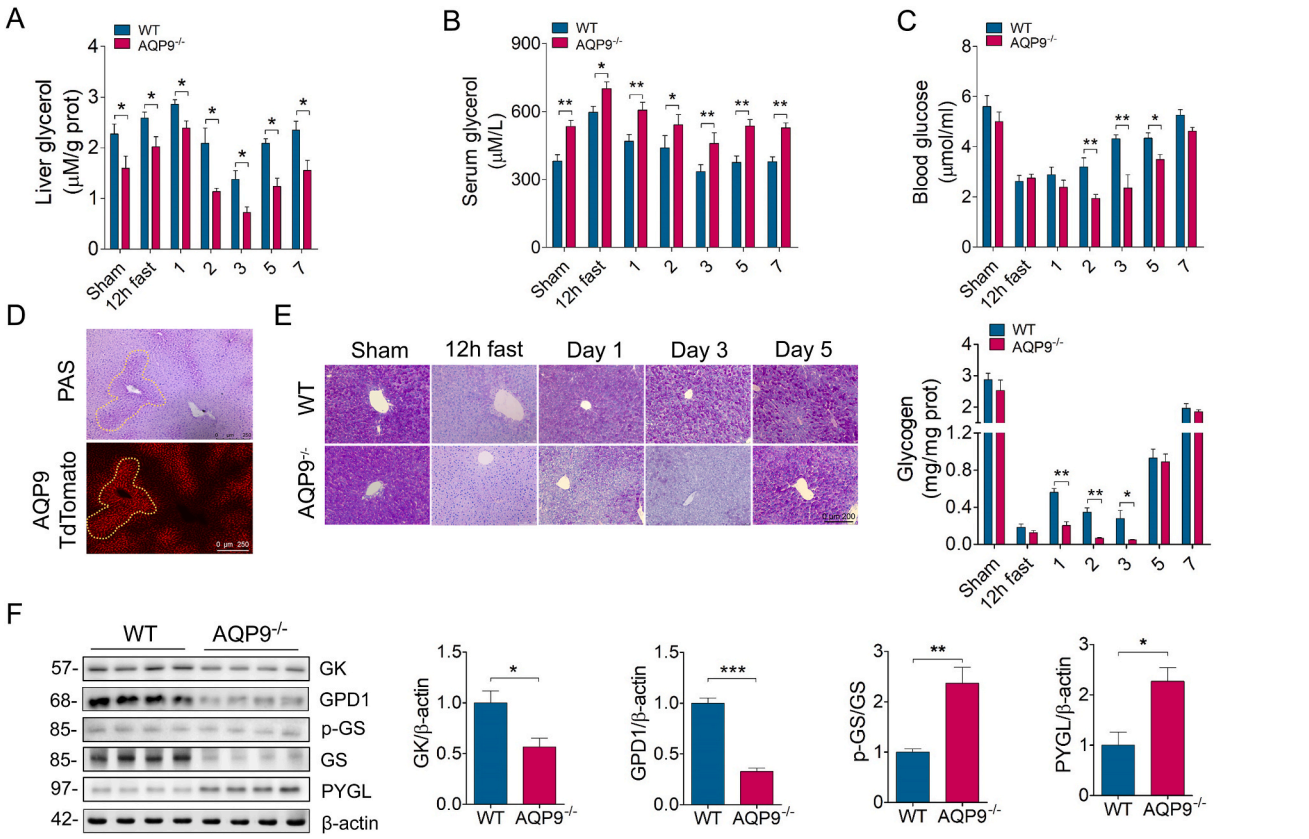

Fig. 4. *AQP9-deficiency impairs liver gluconeogenesis in mice after 70% PH.* Glycerol levels in liver (A) and serum (B) in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated time points (n = 6/group). (C) Blood glucose level in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated time points (n = 6/group). (D) Representative PAS staining and fluorescence images of liver cryosections from AQP9-TdTomato mice. (E) *Left:* PAS staining of liver tissues in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated points (scale bar = 100 μm); *right:* Glycogen content in liver in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at indicated time points (n = 6/group). (F) Western blot analysis of key glycogen synthesis enzymes GK, GPD1, p-GS, GS and glycogenolytic enzyme PYGL in AQP9$^{-/-}$ and AQP9$^{+/+}$ mice at day 3 after 70% PH (n = 4/group). The number on the left of the blot represents the molecular weight of the target protein. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as mean ± S.E.M.

3.5. *Reduced lipolysis in AQP9$^{-/-}$ regenerating liver*

It is well known that major hepatectomy causes a temporary fat accumulation in the liver, which is consumed during liver regeneration [39]. By Oil Red O staining (Supplement Fig. 2A) and biochemical measurements (Supplement Fig. 2B), we observed that liver triglycerides increased significantly after 12 h fasting in both AQP9$^{-/-}$ and AQP9$^{+/+}$ mice. One day after PH triglyceride levels increased remarkably and similarly in livers of AQP9$^{-/-}$ and AQP9$^{+/+}$ mice. However, triglycerides diminished rapidly during regeneration in AQP9$^{+/+}$ liver but remained at high level in AQP9$^{-/-}$ liver. Analysis by quantitative real-time PCR indicated that the expression of lipolysis-related genes, including Atgl and Mgll, were significantly lower in AQP9$^{-/-}$ *vs.* AQP9$^{+/+}$ mice (Supplement Fig. 2C). The lipid droplet formation-related genes Plin2 and lipogenesis-related genes (Fasn and Acaca) did not differ (Supplement Fig. 2D and 2E). These results indicate that fasting and PH-induced fat accumulation in liver is not affected by AQP9 deletion. The reduced liver lipolysis in AQP9$^{-/-}$ mice may be due to decreased fat consumption in the regenerating liver.

3.6. *Rescue of impaired liver regeneration by aquaglyceroporins*

To further investigate the mechanisms of AQP9 in regulating liver regeneration, we generated recombinant AAVs expressing aquaglyceroporins AQP9 and AQP3, and water-selective channel AQP4, under the control of the TBG promoter (Supplement Fig. 3A). Images in Supplement Fig. 3B shows AAV-mediated liver expression of AQP9-GFP, AQP3-GFP or AQP4-GFP in AQP9$^{-/-}$ mice, which was similar to the pattern of AQP9-TdTomato transgenic knock-in mice. We found that AQP9 and AQP3 replacement, but not AQP4 or GFP, rescued the retained liver regeneration (Supplement Fig. 3C) and liver injury (Supplement Fig. 3D) in AQP9$^{-/-}$ mice. Biochemical assays also indicated reduction of hepatocyte H_2O_2 level and increase of liver glycogen content and blood glucose in AQP9$^{-/-}$ mice expressing AQP9-GFP and AQP3-GFP, but not AQP4-GFP after PH (Supplement Fig. 3E and 3F). These results demonstrated that the H_2O_2 and glycerol transporting functions rather than water permeability of AQP9 play key roles in liver regeneration.

In summary, our results support a pivotal role of AQP9 in liver regeneration by reducing oxidative stress by facilitated export of excessive H_2O_2 and maintaining normal gluconeogenesis through efficient uptake of metabolic glycerol in hepatocytes. AQP9 may represent a novel target to enhance liver regeneration after hepatectomy or liver transplantation.

Funding

This work was supported by National Natural Science Foundation of China (Grant number: 82073913).

Declaration of interest

The authors declare that they have no conflicts of interest of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2022.102246.

References

[1] G.K. Michalopoulos, B. Bhushan, Liver regeneration: biological and pathological mechanisms and implications, Nat. Rev. Gastroenterol. Hepatol. 18 (1) (2021 Jan) 40–55, https://doi.org/10.1038/s41575-020-0342-4.

[2] M. Ozaki, Cellular and molecular mechanisms of liver regeneration: proliferation, growth, death and protection of hepatocytes, Semin. Cell Dev. Biol. 100 (2020 Apr) 62–73, https://doi.org/10.1016/j.semcdb.2019.10.007.

[3] C. Mitchell, H. Willenbring, A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice, Nat. Protoc. 3 (7) (2008) 1167–1170, https://doi.org/10.1038/nprot.2008.80.

[4] J. Huang, D.A. Rudnick, Elucidating the metabolic regulation of liver regeneration, Am. J. Pathol. 184 (2) (2014 Feb) 309–321, https://doi.org/10.1016/j.ajpath.2013.04.034.

[5] G.J. DeYulia Jr., J.M. Cárcamo, O. Bórquez-Ojeda, C.C. Shelton, D.W. Golde, Hydrogen peroxide generated extracellularly by receptor-ligand interaction facilitates cell signaling, Proc. Natl. Acad. Sci. U. S. A. 102 (14) (2005 Apr 5) 5044–5049, https://doi.org/10.1073/pnas.0501154102.

[6] R. Singh, M.J. Czaja, Regulation of hepatocyte apoptosis by oxidative stress, J. Gastroenterol. Hepatol. Suppl 22 (2007 Jun) S45–S48, https://doi.org/10.1111/j.1440-1746.2006.04646.x, 1.

[7] F. Böhm, U.A. Köhler, T. Speicher, S. Werner, Regulation of liver regeneration by growth factors and cytokines, EMBO Mol. Med. 2 (8) (2010 Aug) 294–305, https://doi.org/10.1002/emmm.201000085.

[8] R. Zhu, Y. Wang, L. Zhang, Q. Guo, Oxidative stress and liver disease, Hepatol. Res. 42 (8) (2012 Aug) 741–749, https://doi.org/10.1111/j.1872-034X.2012.00996.x.

[9] H. Sies, D.P. Jones, Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, Nat. Rev. Mol. Cell Biol. 21 (7) (2020 Jul) 363–383, https://doi.org/10.1038/s41580-020-0230-3.

[10] L. Zhang, X. Wang, R. Cueto, C. Effi, Y. Zhang, H. Tan, X. Qin, Y. Ji, X. Yang, H. Wang, Biochemical basis and metabolic interplay of redox regulation, Redox Biol. 26 (2019 Sep) 101284, https://doi.org/10.1016/j.redox.2019.101284.

[11] M. Horimoto, P. Fülöp, Z. Derdák, J.R. Wands, G. Baffy, Uncoupling protein-2 deficiency promotes oxidant stress and delays liver regeneration in mice, Hepatology 39 (2) (2004 Feb) 386–392, https://doi.org/10.1002/hep.20047.

[12] G.P. Bienert, F. Chaumont, Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide, Biochim. Biophys. Acta 1840 (5) (2014 May) 1596–1604, https://doi.org/10.1016/j.bbagen.2013.09.017.

[13] M. Elkjaer, Z. Vajda, L.N. Nejsum, T. Kwon, U.B. Jensen, M. Amiry-Moghaddam, J. Frøkiaer, S. Nielsen, Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain, Biochem. Biophys. Res. Commun. 276 (3) (2000 Oct 5) 1118–1128, https://doi.org/10.1006/bbrc.2000.3505.

[14] K. Nihei, Y. Koyama, T. Tani, E. Yaoita, K. Ohshiro, L.P. Adhikary, I. Kurosaki, Y. Shirai, K. Hatakeyama, T. Yamamoto, Immunolocalization of aquaporin-9 in rat hepatocytes and Leydig cells, Arch. Histol. Cytol. 64 (1) (2001 Feb) 81–88, https://doi.org/10.1679/aohc.64.81.

[15] H. Tsukaguchi, S. Weremowicz, C.C. Morton, M.A. Hediger, Functional and molecular characterization of the human neutral solute channel aquaporin-9, Am. J. Physiol. 277 (5) (1999 Nov) F685–F696, https://doi.org/10.1152/ajprenal.1999.277.5.

[16] S. Watanabe, C.S. Moniaga, S. Nielsen, M. Hara-Chikuma, Aquaporin-9 facilitates membrane transport of hydrogen peroxide in mammalian cells, Biochem. Biophys. Res. Commun. 471 (1) (2016 Feb 26) 191–197, https://doi.org/10.1016/j.bbrc.2016.01.153.

[17] R. Solhi, M. Lotfinia, R. Gramignoli, M. Najimi, M. Vosough, Metabolic hallmarks of liver regeneration, Trends Endocrinol. Metabol. 32 (9) (2021 Sep) 731–745, https://doi.org/10.1016/j.tem.2021.06.002.

[18] D.A. Rudnick, N.O. Davidson, Functional relationships between lipid metabolism and liver regeneration, Int J Hepatol 2012 (2012) 549241, https://doi.org/10.1155/2012/549241.

[19] A. Brinkmann, N. Katz, D. Sasse, K. Jungermann, Increase of the gluconeogenic and decrease of the glycolytic capacity of rat liver with a change of the metabolic zonation after partial hepatectomy, Hoppe Seylers Z Physiol Chem. 359 (11) (1978 Nov) 1561–1571, https://doi.org/10.1515/bchm2.1978.359.2.1561.

[20] J. Simek, V. Chmelar, Mělka J, Pazderka, Charvát Z. Influence of protracted infusion of glucose and insulin on the composition and regeneration activity of liver after partial hepatectomy in rats, Nature 213 (5079) (1967 Mar 4) 910–911, https://doi.org/10.1038/213910a0.

[21] E.C. Lin, Glycerol utilization and its regulation in mammals, Annu. Rev. Biochem. 46 (1977) 765–795, https://doi.org/10.1146/annurev.bi.46.070177.004001.

[22] N. Maeda, T. Funahashi, I. Shimomura, Metabolic impact of adipose and hepatic glycerol channels aquaporin 7 and aquaporin 9, Nat. Clin. Pract. Endocrinol. Metabol. 4 (11) (2008 Nov) 627–634, https://doi.org/10.1038/ncpendmet0980.

[23] S. Jelen, S. Wacker, C. Aponte-Santamaría, M. Skott, A. Rojek, U. Johanson, P. Kjellbom, S. Nielsen, B.L. de Groot, M. Rützler, Aquaporin-9 protein is the primary route of hepatocyte glycerol uptake for glycerol gluconeogenesis in mice, J. Biol. Chem. 286 (52) (2011 Dec 30) 44319–44325, https://doi.org/10.1074/jbc.M111.297002.

[24] L. Rui, Energy metabolism in the liver, Compr. Physiol. 4 (1) (2014 Jan) 177–197, https://doi.org/10.1002/cphy.c130024.

[25] A.M. Rojek, M.T. Skowronski, E.M. Füchtbauer, A.C. Füchtbauer, R.A. Fenton, P. Agre, J. Frøkiaer, S. Nielsen, Defective glycerol metabolism in aquaporin 9 (AQP9) knockout mice, Proc. Natl. Acad. Sci. U. S. A. 104 (9) (2007 Feb 27) 3609–3614, https://doi.org/10.1073/pnas.0610894104.

[26] G. Calamita, P. Gena, D. Ferri, A. Rosito, A. Rojek, S. Nielsen, R.A. Marinelli, G. Frühbeck, M. Svelto, Biophysical assessment of aquaporin-9 as principal facilitative pathway in mouse liver import of glucogenetic glycerol, Biol. Cell. 104 (6) (2012 Jun) 342–351, https://doi.org/10.1111/boc.201100061.

[27] A. Rodríguez, P. Gena, L. Méndez-Giménez, A. Rosito, V. Valentí, F. Rotellar, I. Sola, R. Moncada, C. Silva, M. Svelto, J. Salvador, G. Calamita, G. Frühbeck, Reduced hepatic aquaporin-9 and glycerol permeability are related to insulin resistance in non-alcoholic fatty liver disease, Int. J. Obes. 38 (9) (2014 Sep) 1213–1220, https://doi.org/10.1038/ijo.2013.234.

[28] F. Lorenzetti, A.M. Capiglioni, R.A. Marinelli, M.C. Carrillo, M.L. Alvarez, Hepatic glycerol metabolism is early reprogrammed in rat liver cancer development, Biochimie 170 (2020 Mar) 88–93, https://doi.org/10.1016/j.biochi.2020.01.002.

[29] M. Sundaresan, Z.X. Yu, V.J. Ferrans, K. Irani, T. Finkel, Requirement for generation of H2O2 for platelet-derived growth factor signal transduction, Science 270 (5234) (1995 Oct 13) 296–299, https://doi.org/10.1126/science.270.5234.296.

[30] Y.S. Bae, S.W. Kang, M.S. Seo, I.C. Baines, E. Tekle, P.B. Chock, S.G. Rhee, Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation, J. Biol. Chem. 272 (1) (1997 Jan 3) 217–221.

[31] J.R. Stone, S. Yang, Hydrogen peroxide: a signaling messenger, Antioxidants Redox Signal. 8 (3–4) (2006 Mar-Apr) 243–270, https://doi.org/10.1089/ars.2006.8.243.

[32] J.C. Juarez, M. Manuia, M.E. Burnett, O. Betancourt, B. Boivin, D.E. Shaw, N. K. Tonks, A.P. Mazar, F. Doñate, Superoxide dismutase 1 (SOD1) is essential for H2O2-mediated oxidation and inactivation of phosphatases in growth factor signaling, Proc. Natl. Acad. Sci. U. S. A. 105 (20) (2008 May 20) 7147–7152, https://doi.org/10.1073/pnas.0709451105.

[33] L. Conde de la Rosa, M.H. Schoemaker, T.E. Vrenken, M. Buist-Homan, R. Havinga, P.L. Jansen, H. Moshage, Superoxide anions and hydrogen peroxide induce hepatocyte death by different mechanisms: involvement of JNK and ERK MAP kinases, J. Hepatol. 44 (5) (2006 May) 918–929, https://doi.org/10.1016/j.jhep.2005.07.034.

[34] K.C. Hung, P.M. Hsieh, C.Y. Hsu, C.W. Lin, G.M. Feng, Y.S. Chen, C.H. Hung, Expression of aquaporins in rat liver regeneration, Scand. J. Gastroenterol. 47 (6) (2012 Jun) 676–685, https://doi.org/10.3109/00365521.2012.674969.

[35] T.A. Beyer, W. Xu, D. Teupser, U. auf dem Keller, P. Bugnon, E. Hildt, J. Thiery, Y. W. Kan, S. Werner, Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance, EMBO J. 27 (1) (2008 Jan 9) 212–223, https://doi.org/10.1038/sj.emboj.7601950.

[36] H. Kuriyama, I. Shimomura, K. Kishida, H. Kondo, N. Furuyama, H. Nishizawa, N. Maeda, M. Matsuda, H. Nagaretani, S. Kihara, T. Nakamura, Y. Tochino, T. Funahashi, Y. Matsuzawa, Coordinated regulation of fat-specific and liver-specific glycerol channels, aquaporin adipose and aquaporin 9, Diabetes 51 (10) (2002 Oct) 2915–2921, https://doi.org/10.2337/diabetes.51.10.2915.

[37] J. Lebeck, Metabolic impact of the glycerol channels AQP7 and AQP9 in adipose tissue and liver, J. Mol. Endocrinol. 52 (2) (2014 Mar 14) R165–R178, https://doi.org/10.1530/JME-13-0268.

[38] M. Galli, A. Hameed, A. Żbikowski, P. Zabielski, Aquaporins in insulin resistance and diabetes: more than channels!, Redox Biol. 44 (2021 Aug) 102027, https://doi.org/10.1016/j.redox.2021.102027. Epub 2021 May 27. PMID: 34090243; PMCID: PMC8182305.

[39] G.C. Farrell, Probing Prometheus: fat fueling the fire? Hepatology 40 (6) (2004 Dec) 1252–1255, https://doi.org/10.1002/hep.20522.