Molecular Genetics and Metabolism 122 (2017) 95–98

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Brief Communication

Renal endoplasmic reticulum stress is coupled to impaired autophagy in a mouse model of GSD Ia

Benjamin L. Farah[a], Dustin J. Landau[b,c], Yajun Wu[d], Rohit A. Sinha[a], Alwin Loh[e], Boon-Huat Bay[d], Dwight D. Koeberl[b,c], Paul M. Yen[a,f,*]

[a] Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, Singapore, Singapore
[b] Division of Medical Genetics, Department of Pediatrics, Duke University Medical School, Durham, NC, USA
[c] Molecular Genetics and Microbiology, Duke University, Durham, NC, USA
[d] Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[e] Division of Pathology, Singapore General Hospital, Singapore
[f] Duke Molecular Physiology Institute, Duke University School of Medicine, Durham, NC, USA

ARTICLE INFO

Keywords:
Autophagy
Von Gierke's disease
GSD Ia
Rapamycin
Kidney
ER stress
Glucose-6-phosphatase
Glucose-6-phosphate
Glycogen storage disease type I

ABSTRACT

GSD Ia (von Gierke Disease, Glycogen Storage Disease Type Ia) is a devastating genetic disorder with long-term sequelae, such as non-alcoholic fatty liver disease and renal failure. Down-regulated autophagy is involved in the development of hepatic metabolic dysfunction in GSD Ia; however, the role of autophagy in the renal pathology is unknown. Here we show that autophagy is impaired and endoplasmic reticulum (ER) stress is increased in the kidneys of a mouse model of GSD Ia. Induction of autophagy by rapamycin also reduces this ER stress. Taken together, these results show an additional role for autophagy down-regulation in the pathogenesis of GSD Ia, and provide further justification for the use of autophagy modulators in GSD Ia.

1. Introduction

GSD Ia (Glycogen Storage Disease Type Ia, also known as von Gierke disease) is a devastating disorder of glycogen metabolism caused by a loss-of-function of glucose-6-phospatase α encoded by the *G6PC* gene, an enzyme that catalyzes the terminal step of both gluconeogenesis and glycogenolysis, the conversion of glucose-6-phosphate (G6P) to glucose [1]. The failure to generate glucose from the liver leads to hypoglycaemia in infancy that can be prevented by appropriate dietary therapy [1]. However, even when normoglycaemia is maintained, patients develop secondary metabolic sequelae such as non-alcoholic fatty liver disease (NAFLD) and renal failure [1]. Since *G6PC* is primarily expressed in the liver and kidneys, these two tissues are affected most as the disease progresses [1]. In the case of the kidneys, patients with GSD Ia suffer a disease resembling diabetic nephropathy that can progress to renal failure [2].

(Macro)autophagy is a cellular process whereby which damaged or unneeded cellular components are sequestered in organelles called autophagosomes, which are then trafficked to lysosomes for destruction by digestive enzymes [3]. Recently, our group showed that impairment in autophagy is key to the development of hepatic metabolic derangements in this disease [4]. Increased ER stress in neutrophils also has been shown to be pathogenic in the closely-related disease, GSD Ib [5]. In the kidney, autophagy occurred after exposure to insults that caused endoplasmic reticulum (ER) stress [6]. Moreover, treatment with a pharmacological of autophagy, rapamycin, alleviated the effects of ER stressors [7].

Since increased ER stress is a known cause of kidney damage [6], plays a role in the development of podocyte injury [8], as well as mediates the progression of chronic kidney disease in proteinuric mice [9], we hypothesized that increased ER stress may occur in the kidneys of patients suffering from GSD Ia and that treatment with autophagy-inducing agents such as rapamycin might relieve this ER stress. Accordingly, we examined ER stress markers and autophagy in the kidneys of young *G6pc*$^{-/-}$ mice before or after treatment with rapamycin.

Abbreviations: G6Pase α, Glucose-6-phosphatase α; G6PC, Glucose-6-phosphatase α catalytic subunit; GSD Ia, Glycogen Storage Disease Type Ia; G6P, glucose-6-phosphate; LC3, Microtubule-associated protein 1A/1B-light chain 3; Rap, rapamycin; KD, Knockdown; KO, Knockout

* Corresponding author at: Cardiovascular and Metabolic Diseases Program, Duke-NUS Graduate Medical School, 8 College Road, 169547, Singapore.
E-mail address: paul.yen@duke-nus.edu.sg (P.M. Yen).

http://dx.doi.org/10.1016/j.ymgme.2017.08.013
Received 21 June 2017; Received in revised form 23 August 2017; Accepted 24 August 2017
Available online 01 September 2017

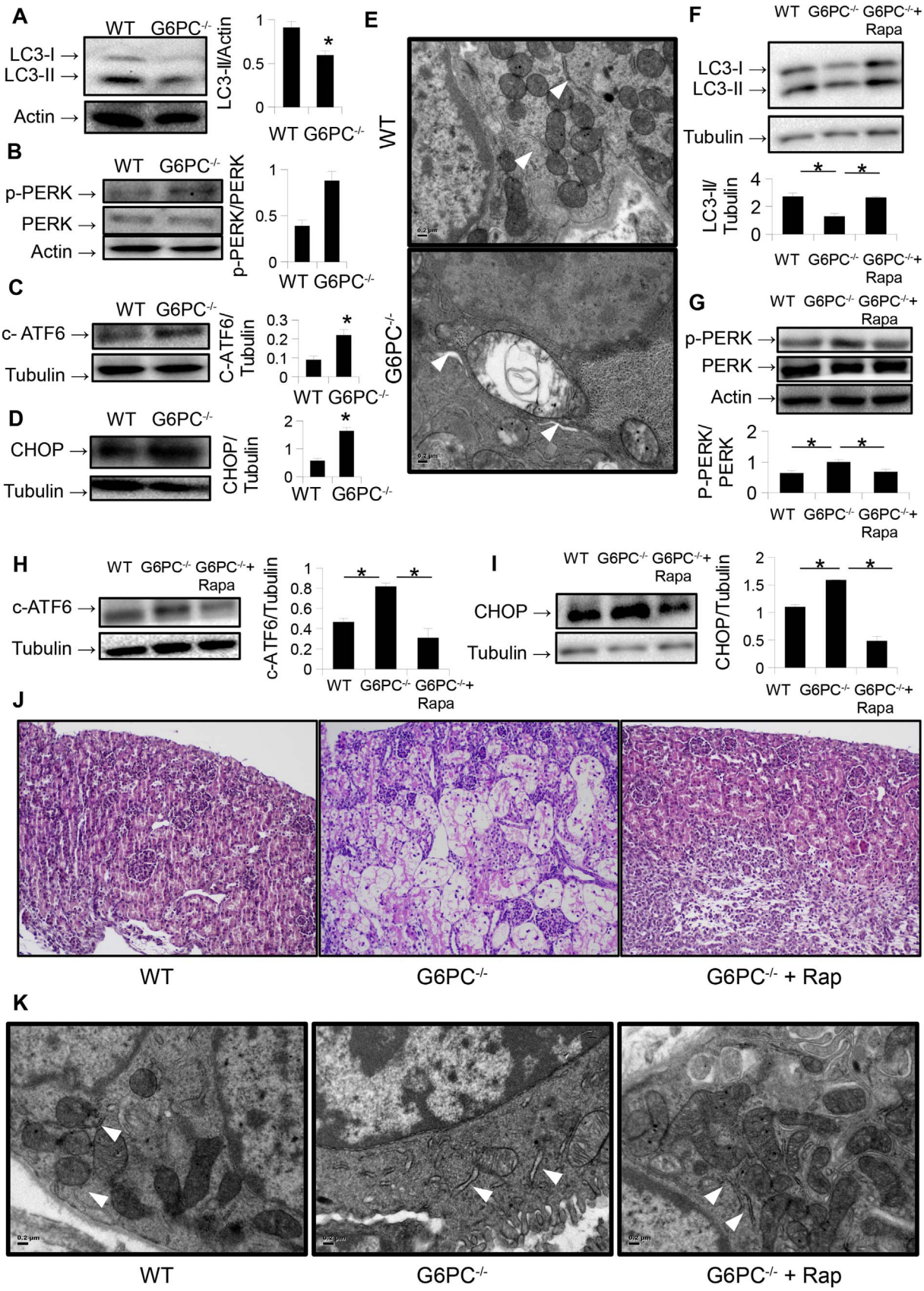

Fig. 1. A.) Levels of autophagosome marker LC3-II are decreased in the kidneys of G6PC$^{-/-}$ mice. B–D.) Protein levels of phosphorylated PERK (B), cleaved ATF6 (c-ATF6) (C), and CHOP (D) are increased in the kidneys of the same mice. E.) Ultrastructural analysis (20,000 ×) of G6PC$^{-/-}$ mouse kidneys reveals dilation of the endoplasmic reticulum (ER). ER noted as white arrowheads. Scale bar = 0.2 μm. F.) Treatment of G6PC$^{-/-}$ mice with rapamycin increases kidney levels of LC3-II. G–I.) Levels of ER stress markers p-PERK (G), cleaved ATF6 (H), and CHOP (I) in these same mice. J.) Histological analysis (20 × objective) with H & E stain in the same mice reveals vacuolation and storage of clear material (lipid/glycogen) in the kidney tubular cells of *G6pc*$^{-/-}$ which resolves with rapamycin treatment. K.) Ultrastructural analysis (20,000 ×) of *G6pc*$^{-/-}$ mouse kidneys following rapamycin treatment reveals and improvement in ER morphology (white arrowheads).

2. Materials and methods

2.1. Study approval

All animal experiments were approved by the Institutional Animal Care and Use Committee of Duke University (protocol number: A070-14-03). All animals received care as per NIH publication 86–23, and all reasonable steps to prevent suffering were undertaken.

2.2. Antibodies

Primary antibodies recognizing phosphorylated-PERK (3179), PERK (3192), CHOP (2895), LC3 (2775), and α -tubulin were purchased from cell signaling technologies. Primary antibody recognizing ATF6 (09-069) was purchased from Merck Millipore. Primary antibody recognizing β-actin (sc-8432) and HRP-conjugated secondary antibodies raised against mouse (sc-2954) and rabbit (sc-2955) IgG were purchased from SantaCruz Biotechnologies.

2.3. Animal models

G6pc$^{-/-}$ mice were identified as described previously [4]. Rapamycin treatment was performed as previously described [4].

2.4. Western blotting

Western blotting was performed identically as per previously described methods [4].

2.5. Electron microscopy

Samples were harvested, prepared and imaged following methods previously published [4].

2.6. Statistics

Animal experiments were performed with 3–5 mice per group. Results are expressed as mean ± SEM. In all parts, * represents $p < 0.05$ as per Student's *t*-test.

2.7. Histology

At sacrifice, kidney tissue was fixed in formalin, then sectioned and stained with haematoxylin and eosin. Images were acquired with a 20 × objective lens (total magnification 200 ×).

3. Results

We first showed that similar to our findings in the liver [4], autophagy was inhibited in the kidneys of *G6pc*$^{-/-}$ mice by Western blotting for the LC3-II levels (Fig. 1A), a commonly used marker for autophagosome number [10]. This decrease in LC3-II was associated with increases in the protein levels of phosphorylated Extracellular Signal-regulated Kinase (pERK), cleaved Activating Transcription Factor 6 (ATF6), and CCAAT-enhancer-binding Protein Homologous Protein (CHOP) (Fig. 1B–D), that are known indicators of ER stress [11]. Ultrastructural analysis also displayed features of ER stress (Fig. 1E), notably dilation of the endoplasmic reticulum [12]. Treatment of these mice with the autophagy-inducing drug rapamycin restored LC3-II levels (Fig. 1F) and reduced the levels of the aforementioned ER stress markers (Fig. 1G–I). Histological analysis revealed cytoplasmic vacuolation of kidney tubular cells in the *G6pc*$^{-/-}$ mice, implying storage of glycogen and lipids, which resolved when treated with rapamycin (Fig. 1J). Rapamycin also reversed the ultrastructural changes observed in the KO mice (Fig. 1K).

4. Discussion

These data provide further new evidence for the importance of autophagy in the pathogenesis of GSD Ia, and extend our previous findings in the liver [4] to the kidneys. By two weeks after birth, levels of renal ER stress makers were elevated in the *G6pc*$^{-/-}$ mice, implying that renal damage already was taking place. Thus, this increase in ER stress may not only serve as an early marker for kidney damage in GSD Ia, but also could lead to the renal failure that eventually occurs. In this connection, a link between ER stress, autophagy, and the development of renal cysts had previously been postulated by Gjorgjieva and colleagues [13]. Our findings here, thus provide evidence to add GSD Ia to the list of many kidney diseases, including diabetic nephropathy [11], in which increased ER stress has been implicated to have a pathogenic role.

Interestingly, treatment with rapamycin for one week reverses many of the changes seen in the ER stress pathway in the kidneys of the *G6pc*$^{-/-}$ mice. We previously showed that induction of autophagy by rapamycin leads to improvement in hepatic metabolism in this disorder [4]. Our current findings show that improvement in autophagy reduces renal ER stress in a mouse model of GSD Ia. Although we did not assess the benefits of long-term treatment with rapamycin on renal function in GSD Ia, our findings nonetheless provide further evidence that autophagy induction may be a viable therapeutic option in GSD Ia.

Conflict of interest

A patent application has been filed by Duke University and Duke-NUS Graduate Medical School related to the treatment of glycogen storage disease with rapamycin, and BLH, PMY, and DDK are inventors.

Financial support

This work was supported by Singapore NMRC grant NMRC/CIRG/1340/2012 and NMRC/CSA/0054/2013 to PMY, as well as by support from the Alice and Y. T. Chen Center for Genetics and Genomics to DDK. This study was also supported by grant R01DK105434 from the National Institute of Diabetes and Digestive and Kidney Diseases. We would also like to acknowledge inspiration and support from Dr. Emory and Mrs. Mary Chapman and their son Christopher, and from Dr. John and Mrs. Michelle Kelly. We deeply appreciate the dedication shown by the staff of the Duke Department of Laboratory Animal Resources.

References

[1] J.Y. Chou, H.S. Jun, B.C. Mansfield, Glycogen storage disease type I and G6pase-beta deficiency: etiology and therapy, Nat. Rev. Endocrinol. 6 (2010) 676–688.
[2] W.H. Yiu, C.J. Pan, R.A. Ruef, W.T. Peng, M.F. Starost, B.C. Mansfield, et al., Angiotensin mediates renal fibrosis in the nephropathy of glycogen storage disease type Ia, Kidney Int. 73 (2008) 716–723.
[3] N. Mizushima, M. Komatsu, Autophagy: renovation of cells and tissues, Cell 147 (2011) 728–741.
[4] B.L. Farah, D.J. Landau, R.A. Sinha, E.D. Brooks, Y. Wu, S.Y. Fung, et al., Induction of autophagy improves hepatic lipid metabolism in glucose-6-phosphatase deficiency, J. Hepatol. 64 (2016) 370–379.
[5] H.S. Jun, Y.M. Lee, Y.Y. Cheung, D.H. McDermott, P.M. Murphy, S.S. De Ravin, et al., Lack of glucose recycling between endoplasmic reticulum and cytoplasm underlies cellular dysfunction in glucose-6-phosphatase-beta-deficient neutrophils in a congenital neutropenia syndrome, Blood 116 (2010) 2783–2792.
[6] A.V. Cybulsky, The intersecting roles of endoplasmic reticulum stress, ubiquitin-proteasome system, and autophagy in the pathogenesis of proteinuric kidney disease, Kidney Int. 84 (2013) 25–33.
[7] G. Dong, Y. Liu, L. Zhang, S. Huang, H.F. Ding, Z. Dong, Mtor contributes to ER stress and associated apoptosis in renal tubular cells, Am. J. Physiol. Ren. Physiol. 308 (2015) F267–274.
[8] Y. Yuan, X. Xu, C. Zhao, M. Zhao, H. Wang, B. Zhang, et al., The roles of oxidative stress, endoplasmic reticulum stress, and autophagy in aldosterone/mineralocorticoid receptor-induced podocyte injury, Lab. Investig. 95 (2015) 1374–1386.
[9] K. El Karoui, A. Viau, O. Dellis, A. Bagattin, C. Nguyen, W. Baron, et al., Endoplasmic reticulum stress drives proteinuria-induced kidney lesions via Lipocalin 2, Nat. Commun. 7 (2016) 10330.
[10] D.J. Klionsky, F.C. Abdalla, H. Abeliovich, R.T. Abraham, A. Acevedo-Arozena,

K. Adeli, et al., Guidelines for the use and interpretation of assays for monitoring autophagy, Autophagy 8 (2012) 445–544.
[11] R. Cunard, K. Sharma, The endoplasmic reticulum stress response and diabetic kidney disease, Am. J. Physiol. Ren. Physiol. 300 (2011) F1054–1061.
[12] T. Asselah, I. Bieche, A. Mansouri, I. Laurendeau, D. Cazals-Hatem, G. Feldmann, et al., In vivo hepatic endoplasmic reticulum stress in patients with chronic hepatitis C, J. Pathol. 221 (2010) 264–274.
[13] M. Gjorgjieva, M. Raffin, A. Duchampt, A. Perry, A. Stefanutti, M. Brevet, et. al, Progressive development of renal cysts in glycogen storage disease type I, Hum. Mol. Genet. 25 (2016) 3784–3797.