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Renal Endoplasmic Reticulum Stress is Coupled to Impaired Autophagy in a Mouse Model of GSD Ia

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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.

Keywords

autophagy; von Gierke's disease; GSD Ia; rapamycin; kidney; ER stress; glucose-6-phosphatase; glucose-6-phosphate; glycogen storage disease type I

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-

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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.

phosphatase α (G6PC), 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 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 an 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.

Materials and Methods

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.

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.

Animal Models

G6PC^{-/-} mice were identified as described previously [4]. Rapamycin treatment was performed as previously described [4].

Western Blotting

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

Electron Microscopy

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

Statistics

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

Histology

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

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. 1 A), 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. 1 B, C, D), that are known indicators of ER stress [11]. Ultrastructural analysis also displayed features of ER stress (Fig. 1 E), notably dilation of the endoplasmic reticulum [12]. Treatment of these mice with the autophagy-inducing drug rapamycin restored LC3-II levels (Fig. 1 F) and reduced the levels of the aforementioned ER stress markers (Fig. 1 G, H, 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. 1 J). Rapamycin also reversed the ultrastructural changes observed in the KO mice (Fig. 1 K).

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 KO 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.

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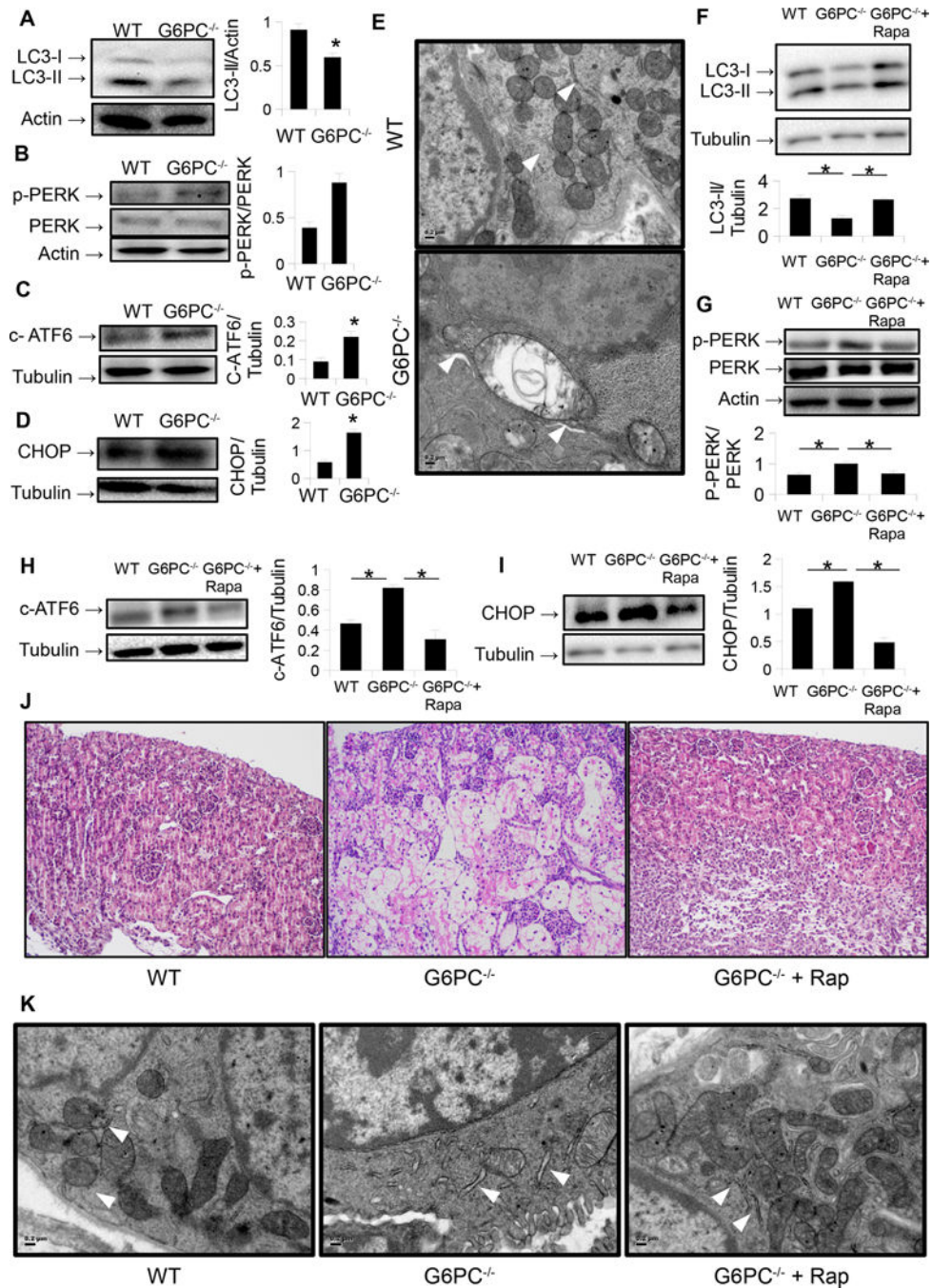
List of Abbreviations

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

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**Figure 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 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 \times) of $G6PC^{-/-}$ mouse kidneys following rapamycin treatment reveals and improvement in ER morphology (white arrowheads).

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