Biochemical and Biophysical Research Communications 459 (2015) 300–305

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Endoplasmic reticulum stress induces up-regulation of hepatic β-Klotho expression through ATF4 signaling pathway

Kun Dong [a], Huating Li [a], Mingliang Zhang [a], Shan Jiang [a], Shuqin Chen [a], Jian Zhou [b], Zhi Dai [b], Qichen Fang [a], Weiping Jia [a, *]

[a] Shanghai Key Laboratory of Diabetes Mellitus, Department of Endocrinology and Metabolism, Shanghai Diabetes Institute, Shanghai Clinical Center for Diabetes, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China
[b] Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Article history:
Received 10 February 2015
Available online 26 February 2015

Keywords:
Endoplasmic reticulum stress
β-Klotho
Fibroblast growth factor 21
Non-alcoholic fatty liver disease

ABSTRACT

Fibroblast growth factor 21 (FGF21) plays critical roles in regulating glucose and lipid metabolism. β-Klotho is the co-receptor for mediating FGF21 signaling, and the mRNA levels of this receptor are increased in the liver of human subjects with obesity. However, the molecular mechanisms underlying the regulation of β-klotho expression remain poorly defined. Here, we report that elevation of β-klotho protein expression in diet-induced obese mice and human patients is associated with increased endoplasmic reticulum (ER) stress. *In vivo* study indicates that administration of the ER stressor tunicamycin in mice led to increased expression of β-klotho in the liver. In addition, we show that ER stress is sufficient to potentiate FGF21 signaling in HepG2 cell and ATF4 signaling pathway is essential for mediating the effect of ER stress on β-klotho expression. These findings demonstrate a link of ER stress with up-regulation of hepatic β-klotho expression and the molecular mechanism underlying ER stress-regulated FGF21 signaling.

1. Introduction

Fibroblast growth factor 21 (FGF21), a member of fibroblast growth factor superfamily, plays critical roles in regulating glucose and lipid homeostasis and energy expenditure *in vivo* [1]. FGF21 acts as a critical regulator of gluconeogenesis, ketogenesis and fatty acid oxidation in liver [2,3], and hepatic FGF21 expression is regulated by fasting, ketogenic diet or activation of PPARα [2,4]. Plasma and hepatic FGF21 was elevated in overweight subjects, impaired glucose tolerance patients or type 2 diabetic patients [5–8]. In white adipose tissue, FGF21 enhances glucose uptake and stimulates lipolysis in insulin-independent manner [1,9,10]. In brown fat tissue, FGF21 promotes thermogenesis, which is partially though PGC-1α [11]. β-Klotho is a co-receptor for FGF21 binding to fibroblast growth factor receptor, which is essential for mediating FGF21 signaling [12,13]. This co-receptor is mainly expressed in liver, pancreas, and adipose tissue [14]. It has been reported that β-klotho is required for the effect of FGF21 on reduction of hepatic triglyceride and cholesterol when mice are challenged with high fat diet [12]. The mRNA expression of β-klotho is elevated in liver in obese human subjects [15], which has been proposed as a compensatory effect to over-nutrition-induced FGF21 resistance [15,16]. However, the molecular mechanisms underlying the regulation of β-klotho expression remain largely unknown.

Endoplasmic reticulum (ER) is the organelle for synthesis, assembly and routing of proteins in cells [17]. The ER stress, also called the unfold protein response (UPR), is due to failure of adaptive capacity between ER protein loading and folding capacity [18]. Numerous studies indicate that ER stress is associated with metabolic diseases including type 2 diabetes and non-alcoholic fatty liver disease (NFALD) [19]. C/EBP homologous protein (CHOP) is considered as the best characterized UPR-regulated protein [20]. Suppression of metabolic gene by CHOP leads to disfunction of fatty acid oxidation, which indicates CHOP may be as a possible mediator of hepatic steatosis [21].

We and others have demonstrated that ER stress induces FGF21 expression in liver by modulating activating transcription factor 4 (ATF4) or X-box-binding protein 1 (XBP1) [22–24]. In present

* Corresponding author. Shanghai Key Laboratory of Diabetes Mellitus, Dept. of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Rd., Shanghai 200233, China. Fax: +86 21 6436 8031.
E-mail address: wpjia@sjtu.edu.cn (W. Jia).

http://dx.doi.org/10.1016/j.bbrc.2015.02.104
0006-291X/

study, we investigate the effects of ER stress on hepatic β-klotho expression and FGF21 signaling. Our results reveal the molecular mechanisms underlying nutrition-regulated hepatic FGF21 signaling.

2. Materials and methods

2.1. Chemicals and antibodies

Thapsigargin (TG) and Tunicamycin (TM) were obtained from Sigma–Aldrich. Anti-Beta-klotho antibody was purchased from R&D Systems. Anti-CHOP (C/EBP homologous protein), anti-phospho-Fra2α, anti-phospho-Erk, and anti-Erk antibodies were purchased from Cell Signaling Technology. Anti–β-actin antibody was obtained from Abcam. Recombinant mouse FGF21 was purchased from Sino Biological.

2.2. Cell culture

Human hepatoma cell line HepG2 (ATCC) was grown in DMEM with 10% fetal bovine serum, 2 mM glutamine, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). To determine the expression levels of β-klotho and CHOP expression under TG or TM stimulation, 80% confluent HepG2 cells were quiescent in DMEM with complete medium followed by treatment with TG (1 μM) or TM (10 ug/ml) for indicated times. To check FGF21 signaling, HepG2 cells were serum-started for 4 h and treated with FGF21 (1 μg/ml) for 15 min.

2.3. Animal studies

2.3.1. Ethics statement

All animal experiments were performed under anesthetic conditions and the protocols were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Shanghai Jiao Tong University.

2.3.2. Mice and method

Male C57BL/6J mice and ob/ob mice were purchased from Shanghai Laboratory Animal Co. Ltd. To generate diet-induced obesity model, mice at 6 weeks old were fed with a high fat diet (HFD) containing 60% kcal of fat or a low fat diet (LFD) containing 10% kcal of fat for 12 weeks (Research Diets Inc.). Male ob/ob mice with the C57BL/6J mice as control were sacrificed at 12 weeks of age. In tunicamycin-induced ER stress study, male C57BL/6J mice at 12 weeks of age under normal chow were injected intraperitoneally with DMSO or tunicamycin at a dose of 1 mg/kg body weight. Twenty-four hours post injection, the mice were sacrificed and the livers were kept in liquid nitrogen before storage at −80 °C.

2.3.3. Primary hepatocyte isolation

Primary hepatocytes were isolated from male mice at 8–12 weeks of age as previously reported [24]. Briefly, the livers were digested with collagenase perfusion through the portal vein of mice, sliced into small pieces in tissue culture dishes with perfusion buffer. The hepatocytes were then filtrated through a 70 μm cell strainer and suspended at 50 g for 2 min at 4 °C. The cells were re-suspended in 15 ml of HepatoZYME- SFM medium supplemented with 2 mM L-glutamine, 20 units/ml penicillin, and 20 ug/ml streptomycin, and cultured for 6 h at 37 °C to adhere before experiments.

2.4. Human liver samples

2.4.1. Ethics statement

The human study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, following the principles of the Declaration of Helsinki. Written voluntary consent was obtained from all subjects before their participation.

2.4.2. Subjects and method

Human liver samples were collected from patients of benign focal hepatic lesions undergoing liver surgery at the Department of Liver Surgery (Zhongshan Hospital, Fudan University, Shanghai, China) as described previously [6] and NAFLD was diagnosed according to the guidelines by the Asia-Pacific Working Party [25]. All liver samples were examined by pathologists and hepatic steatosis was classified as grade 0 (1–5%), grade 1 (6–33%), grade 2 (34–66%), and grade 3 (67–100%) [26]. Liver samples used for protein extraction were from three patients with severe NAFLD (scored as grade 2 or 3) or three control subjects (grade 0). All of these samples were diagnosed without steatohepatitis or cirrhosis.

2.4.3. RNA interference

The RNA interference (siRNA) oligos used to down-regulate *ATF4*, *ATF6*, or *XBP1* expression and scrambled controls were purchased from Shanghai GenePharma Co. Ltd. HepG2 cells were transfected with 20 nM of siRNA or scrambled control by using lipofectamine 2000 (invitrogen). Twenty-four hours post-transfection, the cells were ready for detecting down-regulation of targeted genes.

2.4.4. Western blot analysis

For immunoblotting analysis, total proteins were extracted from cells or liver tissues by RIPA buffer supplemented with protease inhibitor cocktail and phosphatase inhibitors (Sigma). Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Millipore). After incubation with the primary antibodies and then secondary antibodies, proteins were detected with western chemiluminescent HRP substrate (Millipore).

2.4.5. Quantitative real time PCR (RT-PCR) analysis

Total RNAs were extracted from cells or liver tissues by Trizol reagent (Invitrogen). RT-PCR was performed with a Roche Lightcycler 480 system, using the SYBR Green PCR Master Mix (Applied Biosystems). *β-Actin* was used as a control. The primers used for detecting expression of target genes are listed as follows: human *β-klotho*, sense 5′-GGCGACATGGACATTTACATCA-3′ and antisense 5′-ACTTCCGGAGCCGGTCAT-3′; mouse *Chop*, sense 5′-CTGGAAG CCTGGTATGAGGAT-3′ and antisense 5′-CAGGGTCAAGAGTAGTGA AGGT-3′; human *XBP-1s*, sense 5′-CTGAGTCCGAATCAGGTGCAG-3′ and antisense 5′-ATCCATGGGGAGATGTTCTGG-3′; human *CHOP*, sense 5′-CAAGAGGTCCTGTCTTCAGATGA-3′ and antisense 5′-TCTGTTTCCGTTTCCTGGTTC-3′; human *ATF6*, sense 5′-AGCATGTT CCTGAGGAGTTGG-3′ and antisense 5′-AGGCTTATCTTCCTT-CAGTGGC-3′; human *ATF4*, sense 5′-CCTTCGACCAGTCGGGTTTG-3′ and antisense 5′-CTGTCCCGGAAAAGGCATCC-3′; mouse *Ffgr1*, sense 5′-CTGAAGGAGGGTCATCGAAT-3′ and antisense 5′-GTCCAGGTCTTC CACCAACT-3′; mouse *Ffgr2*, sense 5′-CACCACGGACAAAGAGATTG-3′ and antisense 5′-TGTCAACCATGCAGAGTGAA-3′; mouse *Ffgr3*, sense 5′-AGATGCTGAAAGATGATGCG-3′ and antisense 5′-ATGATGTTC TTGTGCTTGCC-3′; mouse *Ffgr4*, sense 5′-CAGAGGCCTTTGGTATG-GAT-3′ and antisense 5′-AGGTCTGCCAAATCCTTGTC-3′; and mouse *β-klotho*, sense 5′-CAGAGAAGGAGGAGGTGAGG-3′ and antisense 5′-CAGCACCTGCCTTAAGTTGA-3′.

2.5. Statistical analysis

All data are presented as mean ± S.E, and p values were calculated using unpaired two-tailed t test (GraphPad Prism 4.0). $p < 0.05$ was considered statistically significant.

3. Results

3.1. ER stress is associated with up-regulation of β-klotho expression in the liver with steatosis

To determine whether hepatic β-klotho expression is associated with ER stress under hepatic steatosis conditions, we developed diet-induced mouse model with obesity and liver steatosis. Our data indicated that HFD-feeding for 12 weeks led to significant increases in body weight (Fig. 1A) and hepatic triglycerides (TG) levels (Fig. 1B) in mice, which is associated with enhancement of β-klotho protein expression (Fig. 1C). Interestingly, levels of CHOP expression, a marker of ER stress [20], in the liver of HFD-fed mice were also significantly increased compared to those fed with chow (Fig. 1C). Similarly, we also observed increased β-klotho and CHOP protein expression in the liver of *ob/ob* mice, a genetic model of obesity [27] (Fig. 1D). To confirm this finding under human liver disease conditions, we detected β-klotho and CHOP protein expression in livers of human NAFLD patients and control subjects. Consistent with the animal study, the expression levels of both β-klotho and CHOP protein were significantly enhanced in the liver of NAFLD patients and control subjects (Fig. 1E). Together, these data indicate that ER stress is associated with up-regulation of β-klotho expression under hepatic steatosis condition in both human and mice.

3.2. ER stress induces up-regulation of β-klotho expression in vitro and in vivo

To investigate whether ER stress has a direct effect on β-klotho expression, we used thapsigargin or tunicamycin, two ER stress

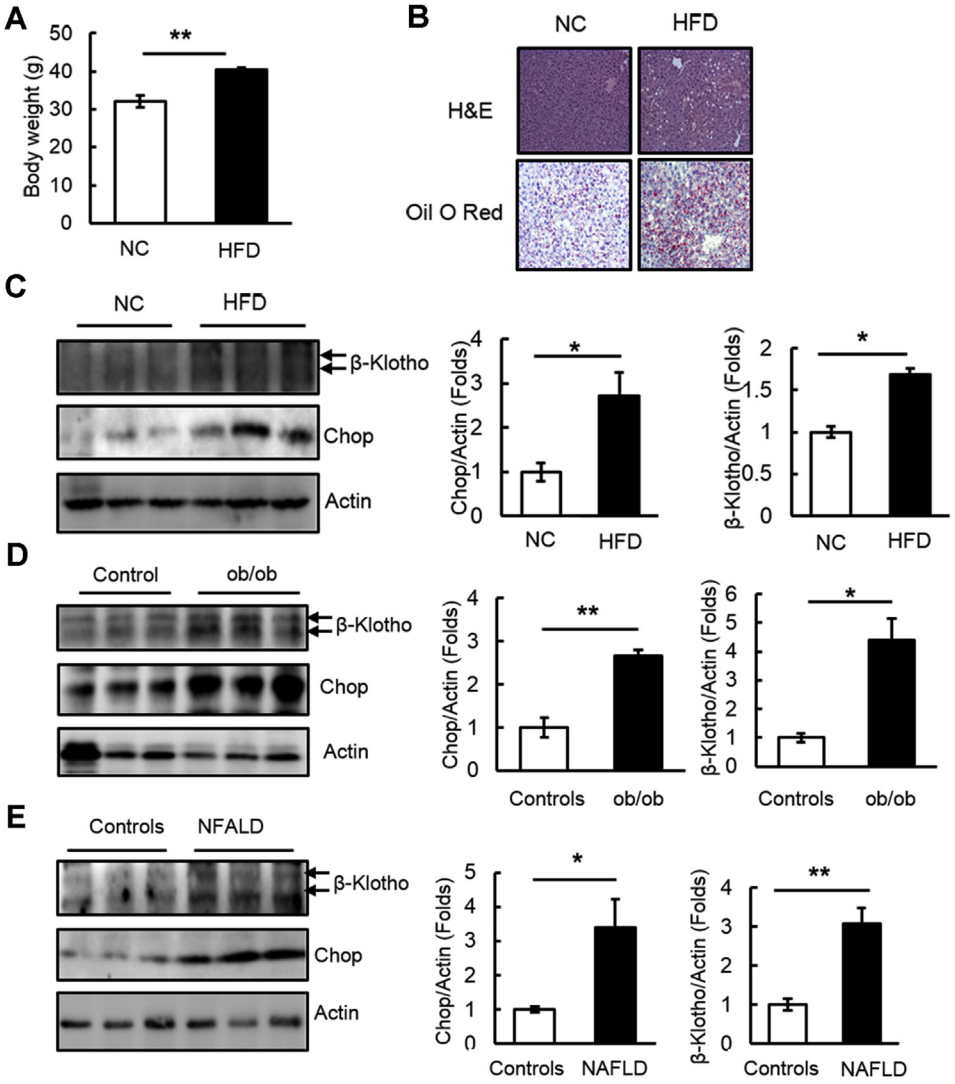

Fig. 1. β-Klotho is elevated in fatty liver. (**A**) Body weight measurements. Male C57BL/6J mice (n = 5) were fed with LFD or HFD at 8 weeks of age for 12 weeks. (**B**) H&E stained and oil O red stained liver sections of the mice fed with LFD or HFD for 12 weeks (20× for each image). (**C**) The liver tissues of the mice fed with LFD or HFD were isolated and homogenized. The protein levels of β-klotho and CHOP were measured by immunoblotting. Actin was used as the loading control, and representative results are shown for three individual mice per group. **(D)** The liver tissues of the *ob/ob* mice and wild type mice were isolated and homogenized. The protein levels of β-klotho and CHOP were measured by immunoblotting. Actin was used as the loading control, and representative results are shown for three individual mice per group. **(E)** The liver tissues of patient with or without NAFLD were extracted and homogenized. The protein levels of β-klotho and CHOP were measured by immunoblotting. Actin was used as the loading control, and representative results are shown for three individual subject per group. Data in A-E are presented as the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$ by unpaired two-tailed t test.

inducers [28], to initiate ER stress in hepatic cells. Our data show that thapsigargin or tunicamycin treatment induced ER stress in HepG2 cells since CHOP expression was significantly increased under these conditions (Fig. 2A and B). Meanwhile, β-klotho expression in both mRNA (Fig. 2C and D) and protein (Fig. 2A and B) levels were up-regulated under these condition. Similar results were observed in mice primary hepatocytes stimulated with tunicamycin (Fig. 2E). To test if ER stress can regulate β-klotho expression *in vivo*, we administrated C57BL/6J male mice with tunicamycin for 24 h. Our data indicate that Tunicamycin was sufficient to induce ER stress in the liver tissues of the mice since the protein (Fig. 2F) and mRNA (Fig. 2G) levels of CHOP expression were markedly enhanced under Tunicamycin treatment. Consistent with our finding that ER stress induces up-regulation of β-klotho expression in both liver cells and primary hepatocyte (Figs. 1 and 2), the levels of both β-klotho protein (Fig. 2F) and mRNA (Fig. 2H) were significantly increased in the liver of mice treated with Tunicamycin compared to those with vehicle control. It has been reported that β-klotho-mediated FGF21 signaling is depended on FGFR1-4 [29,30], receptors of fibroblast growth factor. We next tested the effect of ER stress on these receptors. Our data indicated that ER stress selectively induced down-regulation of *FGFR3*, but not *FGFR1*, *FGFR2*, and *FGFR4* (Fig. 2I), in the liver of Tunicamycin-treated mice, which is opposite to the effect on β-klotho expression (Fig. 2A and B). Taken together, these data demonstrate that the effect of ER stress on β-klotho expression *in vitro* and *in vivo*.

3.3. ATF4 signaling pathway mediates the effect of ER-stress on β-klotho expression

To understand the mechanism underlying ER stress-induced up-regulation of β-klotho expression, we detected if ATF4, ATF6 or XBP1s signaling pathways, which have been reported as ER stress activated pathways in liver [21], are involved in mediating the effect of ER stress on β-klotho expression. The expression of ATF4, activating transcription factor 6 (ATF6), or XBP1s was significantly down regulated with siRNA method in HepG2 cells (Fig. 3A). Interestingly, suppression of ATF4, but not ATF6, or XBP1s, blocked ER-stress-induced β-klotho expression (Fig. 3B). The role of ATF4 in mediating ER-stress effect on β-klotho protein expression was also confirmed (Fig. 3C). These data indicate ER stress-induced β-klotho expression is dependent on ATF4 pathway.

3.4. ER stress stimulates basal level of FGF21 pathway

Due to β-klotho and FGF21 expression can be induced by ER stress [23,24], it is interesting to test whether FGF21 signaling is

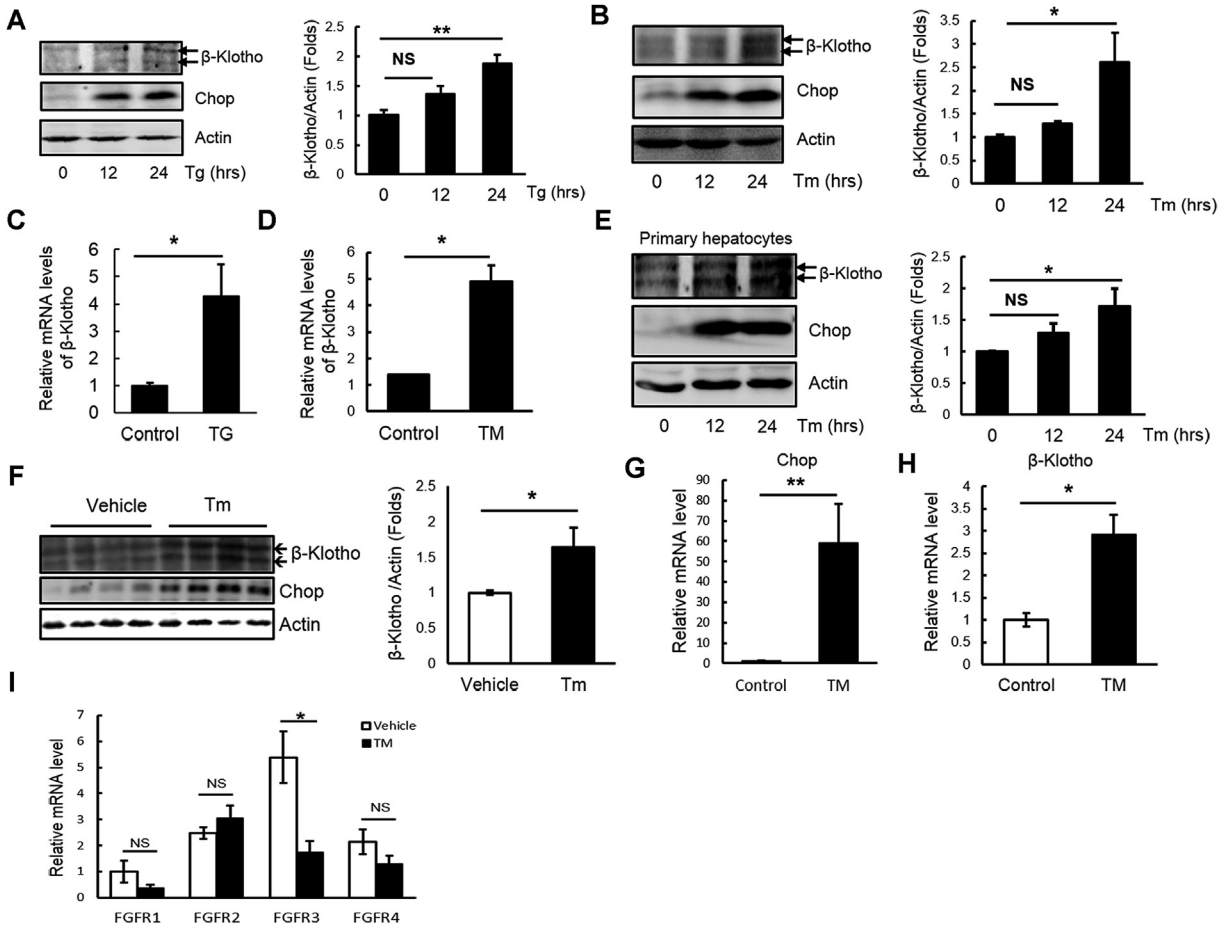

Fig. 2. ER stress induces up-regulation of β-klotho expression *in vitro* and *in vivo*. (**A**) **and** (**B**) HepG2 cells were treated with TG (1uM) or TM (10ug/ml) for indicated time. The protein expression of β-klotho and CHOP were detected by immunoblotting. Shown are representative results from three independent experiments. **(C)** and **(D)** HepG2 cells were treated with TG (1uM) or TM (10ug/ml) for 24 h. The mRNA level of *β-klotho* were measured by RT-PCR. **(E)** Mouse primary hepatocytes were treated with TM (10 μg/ml) for indicated time. The protein expression of β-klotho and CHOP were detected by immunoblotting. Mice were treated with PBS (vehicle) or Tm for 24 h (n = 5/group). (**F**) Hepatic CHOP and β-klotho protein level were measured by immunoblotting. Relative level of β-klotho are shown from densitometric quantification of the immunoblots after normalization to actin. The mRNA levels of *CHOP* **(G)** and *β-klotho* **(H)** were detected by RT-PCR in the liver of Tm-treated mice. **(I)** The mRNA levels of *FGFR1*, *FGFR2*, *FGFR3* and *FGFR4* were measured by RT-PCR in the liver of Tm-treated mice. Data in A–D are shown as the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$ by unpaired two-tailed t test.

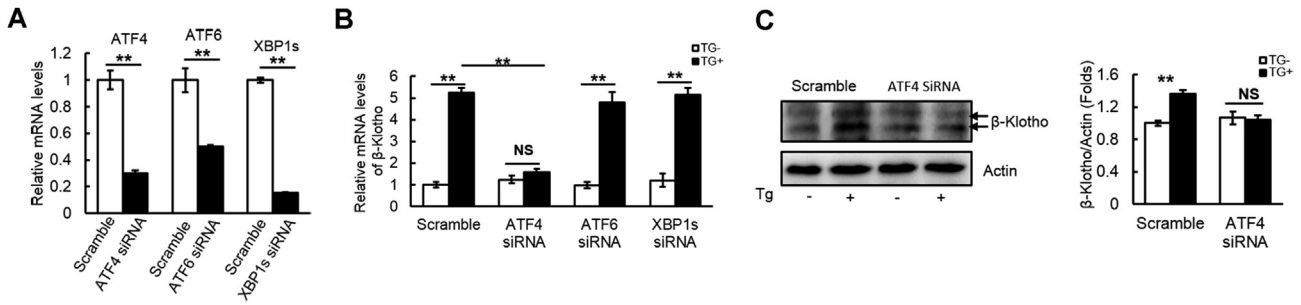

Fig. 3. ER stress increases β-klotho though ATF4 pathway. (**A**) HepG2 cells were transfected with ATF4, ATF6 or XBP1s siRNA for 24 h. The mRNA abundance of *ATF4*, *ATF6* and *XBP1s* were measured by RT-PCR. (**B**) HepG2 cells were transfected with ATF4, ATF6 or XBP1s siRNA for 24 h, then treated with TG (1 uM) or PBS for 24 h. The mRNA abundance of *β-klotho* was measured by RT-PCR. (**C**) HepG2 cells were transfected with ATF4 siRNA for 24 h, then treated with TG (1uM) for 24 h. β-klotho protein expression was measured by immunoblotting. Shown are representative results from three independent experiments. A and B are shown as the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$ by unpaired two-tailed t test.

enhanced under ER stress. HepG2 cells were pre-treated with tunicamycin for 24 h, and then treated with recombinant FGF21. As shown in Fig. 4, ER stress led to increase in basal phosphorylation levels of P-FRS2α and P-Erk, two FGF21 signaling markers [30,31], suggesting that ER stress is sufficient to enhance basal level of FGF21 signaling.

4. Discussion

β-Klotho is a co-receptor of FGF21 and expresses mainly in WAT, BAT, and liver tissues [32], the target organs of FGF21 action [4,12,31,32]. Although β-klotho is essential for beneficial roles of FGF21, increased hepatic mRNA expression of *β-klotho* is related to human obesity [15]. It is largely unknown how β-klotho expression is up-regulated in obesity. In this study, we determine that elevated protein level of β-klotho are associated with ER stress under NAFLD conditions in both mice and humans. We also demonstrate that ER stress can induce up-regulation of β-klotho expression both *in vivo* and *in vitro*. Furthermore, our data indicate that ER stress promotes FGF21 signaling and ATF4 signal pathway mediates the role of ER stress in hepatic cells.

ER stress is closely linked to obesity and type 2 diabetes [18]. Several studies have demonstrated that hepatic FGF21 expression is induced by ER stress through PERK-ATF4 and IRE1-XBP1 pathway [22–24]. However, the effects of ER stress on FGF21 receptor/co-receptor expression and signaling in liver are largely unknown. Previous study indicates that ER stress can induce down-regulation of β-klotho in adipose tissue [33]. Interestingly, our data show for the first time that induction of ER stress with tunicamycin or thapsigargin leads to significant up-regulation of β-klotho expression in both HepG2 cells and mouse primary hepatocytes, indicating the direct effects of ER stress on this co-receptor. We also demonstrate the causal effect of ER stress on up-regulation of hepatic β-klotho expression in both mRNA and protein levels *in vivo*. The correlation of ER stress with increased β-klotho expression was also confirmed in the livers of obese mice and NAFLD patients. Enhanced FGF21 expression and hepatic β-klotho has been showed under over-nutrition condition [15,16]. Our findings that up-regulated hepatic β-klotho and its correlation with ER stress under obesity and NAFLD conditions suggest the potential role of ER stress in regulating FGF21 signaling and function.

In addition to β-klotho, several forms of FGFR including FGFR1-4 have been reported as FGF21 receptors [13,29,30,34]. In contrast to β-klotho, our study indicates that ER stress resulted in down-regulation of FGFR3 and this effect was selective among FGFR family members. Although FGF21 shows high binding affinity to FGFR1 compared to other FGFR isoforms [35], it has been reported that FGF21 can also activate other FGFR isotypes including FGFR3 [34]. Down-regulation of FGFR3 by ER stress may contribute to impairment of FGF21 signaling in liver.

Impaired adaptive capacity of ER leads to activation of the unfolded protein response (UPR). Under ER stress conditions, PERK-ATF4, IRE1-XBP1, and ATF6 are activated and mediate ER stress signaling. In our study, we determined the roles of these pathways in mediating ER stress action by using siRNA approach. Our results indicate that ATF4, but not XBP1s or ATF6, regulated β-klotho expression in response to ER stress. Further study needs to test the molecular mechanisms underlying the role of ATF4 pathway in regulating β-klotho expression.

FGF21 signaling is also mediated by FRS2α and ERK phosphorylation [13,31]. Our data indicate that ER stress is sufficient to enhance the basal levels of FRS2α and ERK phosphorylation. FGF21 treatment cannot further stimulate the phosphorylation, which is probably due to the fact that basal phosphorylation of these molecules were saturated under this condition.

In summary, our findings reveal the association of ER stress with enhanced hepatic β-klotho expression under hepatic steatosis condition. We have also identified ATF4 signaling pathway that

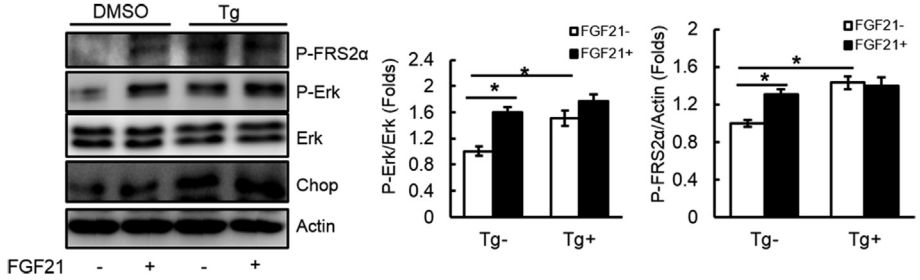

Fig. 4. ER stress increases basal FGF21 signaling. HepG2 cells were treated with TG (1 uM) for 24 h, then treated with FGF21 (1 ug/ml) after 4 h fasting. P-FRS2α, P-ERK and ERK were detected by immunoblotting. Shown are representative results from three independent experiments. Ratios of P-ERK/ERK were quantified by densitometry from the immunoblots. Relative P-FRS2α levels were normalized to actin. Data are shown as the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$ by unpaired two-tailed t test.

mediates the action of ER stress on β-klotho expression in liver. Our finding uncovers a mechanism underlying overnutrition-induced alteration of FGF21 signaling and function.

Conflict of interest

The authors have declared that no competing interests exist.

Acknowledgments

This work was supported by the National 973 project of China (2011CB504001), the National Nature Science Foundation of China (81170379), and the Shanghai Natural Science Foundation (15ZR1431700).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.104.

References

[1] A. Kharitonenkov, T.L. Shiyanova, A. Koester, et al., FGF-21 as a novel metabolic regulator, J. Clin. Invest 115 (2005) 1627–1635.
[2] T. Inagaki, P. Dutchak, G. Zhao, et al., Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21, Cell. Metab. 5 (2007) 415–425.
[3] M.J. Potthoff, T. Inagaki, S. Satapati, et al., FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response, Proc. Natl. Acad. Sci. U S A 106 (2009) 10853–10858.
[4] M.K. Badman, P. Pissios, A.R. Kennedy, et al., Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states, Cell. Metab. 5 (2007) 426–437.
[5] H. Li, K. Dong, Q. Fang, et al., High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China, J. Hepatol. 58 (2013) 557–563.
[6] H. Li, Q. Fang, F. Gao, et al., Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride, J. Hepatol. 53 (2010) 934–940.
[7] M. Mraz, M. Bartlova, Z. Lacinova, et al., Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity, Clin. Endocrinol. (Oxf) 71 (2009) 369–375.
[8] A.O. Chavez, M. Molina-Carrion, M.A. Abdul-Ghani, et al., Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance, Diabetes Care 32 (2009) 1542–1546.
[9] W. Chen, R.L. Hoo, M. Konishi, et al., Growth hormone induces hepatic production of fibroblast growth factor 21 through a mechanism dependent on lipolysis in adipocytes, J. Biol. Chem. 286 (2011) 34559–34566.
[10] Y. Hotta, H. Nakamura, M. Konishi, et al., Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver, Endocrinology 150 (2009) 4625–4633.
[11] F.M. Fisher, S. Kleiner, N. Douris, et al., FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis, Genes. Dev. 26 (2012) 271–281.
[12] X. Ding, J. Boney-Montoya, B.M. Owen, et al., betaKlotho is required for fibroblast growth factor 21 effects on growth and metabolism, Cell. Metab. 16 (2012) 387–393.
[13] Y. Ogawa, H. Kurosu, M. Yamamoto, et al., BetaKlotho is required for metabolic activity of fibroblast growth factor 21, Proc. Natl. Acad. Sci. U S A 104 (2007) 7432–7437.
[14] S. Ito, S. Kinoshita, N. Shiraishi, et al., Molecular cloning and expression analyses of mouse betaklotho, which encodes a novel klotho family protein, Mech. Dev. 98 (2000) 115–119.
[15] J.M. Gallego-Escuredo, J. Gomez-Ambrosi, V. Catalan, et al., Opposite alterations in FGF21 and FGF19 levels and disturbed expression of the receptor machinery for endocrine FGFs in obese patients, Int. J. Obes. (Lond) 39 (2015) 121–129.
[16] F.M. Fisher, P.C. Chui, P.J. Antonellis, et al., Obesity is a fibroblast growth factor 21 (FGF21)-resistant state, Diabetes 59 (2010) 2781–2789.
[17] I. Braakman, D.N. Hebert, Protein folding in the endoplasmic reticulum, Cold Spring Harb. Perspect. Biol. 5 (2013) a013201.
[18] G.S. Hotamisligil, Endoplasmic reticulum stress and the inflammatory basis of metabolic disease, Cell 140 (2010) 900–917.
[19] L. Ozcan, I. Tabas, Role of endoplasmic reticulum stress in metabolic disease and other disorders, Annu Rev. Med. 63 (2012) 317–328.
[20] X.Z. Wang, B. Lawson, J.W. Brewer, et al., Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153), Mol. Cell. Biol. 16 (1996) 4273–4280.
[21] D.T. Rutkowski, J. Wu, S.H. Back, et al., UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators, Dev. Cell. 15 (2008) 829–840.
[22] F.G. Schaap, A.E. Kremer, W.H. Lamers, et al., Fibroblast growth factor 21 is induced by endoplasmic reticulum stress, Biochimie 95 (2013) 692–699.
[23] X.S. Wan, X.H. Lu, Y.C. Xiao, et al., ATF4- and CHOP-dependent induction of FGF21 through endoplasmic reticulum stress, Biomed. Res. Int. 2014 (2014) 807874.
[24] S. Jiang, C. Yan, Q.C. Fang, et al., Fibroblast growth factor 21 is regulated by the IRE1alpha-XBP1 branch of the unfolded protein response and counteracts endoplasmic reticulum stress-induced hepatic steatosis, J. Biol. Chem. 289 (2014) 29751–29765.
[25] D.E. Kleiner, E.M. Brunt, M. Van Natta, et al., Design and validation of a histological scoring system for nonalcoholic fatty liver disease, Hepatology 41 (2005) 1313–1321.
[26] S. Chitturi, G.C. Farrell, E. Hashimoto, et al., Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines, J. Gastroenterol. Hepatol. 22 (2007) 778–787.
[27] A. Chatzigeorgiou, A. Halapas, K. Kalafatakis, et al., The use of animal models in the study of diabetes mellitus, In Vivo 23 (2009) 245–258.
[28] U. Ozcan, Q. Cao, E. Yilmaz, et al., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science 306 (2004) 457–461.
[29] R. Goetz, M. Ohnishi, X. Ding, et al., Klotho coreceptors inhibit signaling by paracrine fibroblast growth factor 8 subfamily ligands, Mol. Cell. Biol. 32 (2012) 1944–1954.
[30] H. Kurosu, M. Choi, Y. Ogawa, et al., Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21, J. Biol. Chem. 282 (2007) 26687–26695.
[31] A. Kharitonenkov, J.D. Dunbar, H.A. Bina, et al., FGF-21/FGF-21 receptor interaction and activation is determined by betaKlotho, J. Cell. Physiol. 215 (2008) 1–7.
[32] F.M. Fisher, J.L. Estall, A.C. Adams, et al., Integrated regulation of hepatic metabolism by fibroblast growth factor 21 (FGF21) in vivo, Endocrinology 152 (2011) 2996–3004.
[33] J. Diaz-Delfin, E. Hondares, R. Iglesias, et al., TNF-alpha represses beta-klotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway, Endocrinology 153 (2012) 4238–4245.
[34] M. Suzuki, Y. Uehara, K. Motomura-Matsuzaka, et al., betaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c, Mol. Endocrinol. 22 (2008) 1006–1014.
[35] C. Yang, C. Jin, X. Li, et al., Differential specificity of endocrine FGF19 and FGF21 to FGFR1 and FGFR4 in complex with KLB, PLoS One 7 (2012) e33870.