Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Basic Science

# Reduced expression of ERp46 under diabetic conditions in β-cells and the effect of liraglutide

*Eugenia Lampropoulou, Anna Lymperopoulou, Aristidis Charonis**

*Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Soranou Efessiou 4, Athens 115 27, Greece*

ARTICLE INFO

*Article history:*
Received 11 July 2015
Accepted 12 September 2015

*Keywords:*
Endoplasmic reticulum protein 46
Glucose toxicity
Proinsulin
Liraglutide
Activating transcription factor 6
X-box binding protein 1

ABSTRACT

*Background.* Diabetes mellitus is characterized by peripheral insulin resistance, hyperglycemia and defective insulin secretion. Insulin producing pancreatic β-cells are equipped with a highly developed endoplasmic reticulum (ER) and thus are affected by ER stress under hyperglycemic conditions. We have previously studied the influence of high glucose on cultured β-cells *in vitro*. Proteomic analysis revealed a number of proteins involved in glucose toxicity, while further biochemical analysis identified the endoplasmic reticulum protein ERp46 as a molecule with a possible role in insulin production at the post-translational level. In addition, the involvement of incretin hormone glucagon-like peptide 1 (GLP-1) in diabetes proposes that incretin-mimetic compounds may be among the optimal choices in future therapeutic interventions; therefore their effects on various aspects of the pathogenesis of diabetes mellitus should be explored in detail. Based on the above, we examined the possible involvement of ERp46 in insulin production and the effect of the GLP-1 analogue liraglutide on the expression of ERp46 *in vitro*, in β-cells cultured under high glucose conditions and *in vivo*, in the mouse *db/db* diabetic model, where pronounced hyperglycemia is a key characteristic.

*Results.* Confocal microscopy revealed areas of co-localization of ERp46 and pro-insulin in pancreatic islets. In order to explore the possible interaction between ERp46 and insulin immunoprecipitation was used. In extracts from cultured β-cells, antibodies against pro-insulin co-precipitated ERp46 and antibodies against ERp46 co-precipitated pro-insulin, as shown by Western blotting. Furthermore, data from a proximity ligation assay positioned these two molecules closer than 30 nm in distance. When pancreatic β-cells were cultured in high glucose conditions they exhibited a decrease in ERp46 expression, while treatment with the GLP-1 analogue liraglutide restored ERp46 levels, leading to a significant increase of ERp46 in comparison to hyperglycemic conditions. In the diabetic mouse model *db*$^{-}$*/db*, ERp46 expression was reduced in pancreatic islets, as documented by morphological and biochemical techniques. This decrease was abolished after treatment with the GLP-1 analogue in a dose-dependent manner. In an attempt to understand the underlying mechanism, we examined the sequence of the promoter of ERp46 and found consensus motifs that can be recognized by transcription factors ATF6 and XBP1. Subsequently, we performed chromatin immunoprecipitation assay and demonstrated that treatment of β-TC-6 cells with 25 mmol/L glucose decreases gradually the binding enrichment of ATF6 and XBP1 in ERp46 gene promoter.

* *Corresponding author*. Tel.: +30 210 6597205; fax: +30 210 6597545.
E-mail addresses: eugenialamprop@gmail.com (E. Lampropoulou), anna_lybero@hotmail.com (A. Lymperopoulou), acharonis@bioacademy.gr (A. Charonis).

http://dx.doi.org/10.1016/j.metabol.2015.09.011

*Conclusions*. We propose that since ERp46 is a member of the disulfide isomerases family, it is likely to play a key role in insulin biosynthesis and its reduction under high glucose conditions may be a novel contributor to the glucotoxicity of β-cells. In addition, the GLP-1 analogue liraglutide seems to interfere in this process and may exert its beneficial effects in diabetes by affecting insulin production via restoration of ERp46 expression.



## 1. Introduction

Diabetes mellitus (DM) is considered the most common metabolic disease with a rising incidence. According to the World Health Organization, over 300 million people worldwide suffer from diabetes, while type 2 diabetes (T2D) accounts for 90% of diabetics [1]. T2D is characterized by hyperglycemia due to defects in insulin production and secretion from pancreatic β-cells or insulin action on target tissues [2]. Hyperglycemia leads to glucose toxicity for pancreatic β-cells [3]. Pancreatic β-cells have a highly developed endoplasmic reticulum (ER) as a key feature, making them more vulnerable to ER stress under hyperglycemic conditions, where ER homeostasis is disrupted. In that case the ER triggers the unfolded protein response (UPR), which consists of adaptive signaling pathways, in order to maintain the homeostasis of ER. However, if homeostasis fails to be restored, the ER initiates death signaling pathways [4].

It is well established that deficient incretin secretion may play a critical role in the pathogenesis of type 2 diabetes and incretin-mimetic compounds may be among the optimal choices in future therapeutic interventions [5]. Therefore their effects on various aspects of the pathogenesis of diabetes mellitus should be the focus of research and further explored in detail. Liraglutide, a long acting GLP-1 receptor agonist, is prescribed for the treatment of TIID, administered once daily and is also approved as monotherapy for obesity and weight control [5]. The efficacy of this compound has been demonstrated in large, controlled studies in terms of the drug's low risk for hypoglycemia when used as monotherapy, HbA1c reduction, and reductions in body weight [6].

Understanding the factors involved and the mechanisms operating at the molecular level and contributing to glucose toxicity is a crucial aspect of diabetes research. Since this is a multifactorial phenomenon, several groups have employed system biology approaches for this purpose [3]. Using proteomic analysis in cultured β-cells, our group has provided evidence in the past that ERp46 is involved in the phenomenon of glucose toxicity [7]. ERp46 is an endoplasmic reticulum resident protein with possible involvement in formation, reduction and isomerization of disulfide bonds in polypeptides. More specifically, we have observed a reduction in ERp46 expression under high glucose conditions and a possible role in insulin production [7].

In this study, **first**, we provide evidence that ERp46 is closely associated and interacting with pro-insulin; **second** that the reduction of ERp46 under high glucose conditions (both in cultured β-cells and in an animal model of diabetes) is restored by liraglutide and **third**, that transcription factors that contribute to UPR may be involved in the differential expression of ERp46 under high glucose conditions. These findings propose a novel pathogenetic pathway under hyperglycemic conditions in which ERp46 plays a crucial role and point to new therapeutic targets.

## 2. Experimental Procedures

### 2.1. *Cell Culture*

β-TC-6 cells (ATCC) were grown in DMEM culture medium containing 15% FBS, 2 mmol/L glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 °C. The medium was changed every 48 h, and cells were passaged once weekly using standard trypsin-EDTA concentrations. Beginning at passage 32, β-TC-6 cells were cultured continuously in either 5 (normal conditions) or 25 mmol/L glucose (hyperglycemic conditions) [7].

### 2.2. *In Vivo Analysis*

The mouse model of $db^-/db^-$ was used in *in vivo* studies (C57BLK) [8]. 10-Week-old male C57BL/6 mice were purchased from Taconic Biosciences and housed for 2 weeks in our Center for Experimental Surgery. After 2 weeks blood glucose levels (mg/dl) and weight (g) were measured for all animals: heterozygous mice ($db^+/db^-$) weighing between 26 and 31 g and had glucose levels between 120 and 200 mg/dl, while homozygous ($db^-/db^-$) with a weight between 45 and 52 g showed pronounced hyperglycemia and the levels of blood glucose were >600 mg/dl. Animals were divided into three groups: 12 ($db^-/db^-$) and 8 ($db^+/db^-$) animals were injected with PBS and used as control, 12 ($db^-/db^-$) and 8 ($db^+/db^-$) animals were injected with 200 μg liraglutide (Novo Nordisk)/kg of body weight and 12 ($db^-/db^-$) and 8 ($db^+/db^-$) animals were injected with 1000 μg liraglutide/kg of body weight (Novo Nordisk). All injections were administered subcutaneously, every day, for 2 weeks. Animals were euthanized and pancreata were isolated for further analysis.

### 2.3. *Immunoprecipitation (IP) Assay*

Cells were lysed with RIPA buffer, as previously described [9,10]. Briefly, when cells were grown to 90% confluence, the medium was aspirated; cells were washed twice with ice-cold PBS and lysed with PBS containing 1% Triton X-100, 0.1% SDS, 1 mmol/L phenylmethylsulfonyl fluoride, and 5 mmol/L EDTA. Cells were scraped off the plate, transferred to Eppendorf tubes, kept on ice for 30 min, and centrifuged at 20,000*g* for 30 min at 4 °C. One to three milligrams of supernatant was transferred to new Eppendorf tubes and incubated with primary antibody for 16 h at 4 °C under