Biochimica et Biophysica Acta 1861 (2016) 1893–1910

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Glucolipotoxicity diminishes cardiomyocyte TFEB and inhibits lysosomal autophagy during obesity and diabetes

Purvi C. Trivedi MSc [a], Jordan J. Bartlett BSc [a], Lester J. Perez PhD [a], Keith R. Brunt PhD [b], Jean Francois Legare MD MSc [c], Ansar Hassan MD PhD [c], Petra C. Kienesberger PhD [a], Thomas Pulinilkunnil PhD [a,*]

[a] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Dalhousie University, Dalhousie Medicine New Brunswick, 100 Tucker Park Road, Saint John E2L4L5, New Brunswick, Canada
[b] Deparment of Pharmacology, Faculty of Medicine, Dalhousie University, Dalhousie Medicine New Brunswick, 100 Tucker Park Road, Saint John E2L4L5, New Brunswick, Canada
[c] Department of Surgery, Faculty of Medicine, Dalhousie University, Dalhousie Medicine New Brunswick, 100 Tucker Park Road, Saint John E2L4L5, New Brunswick, Canada

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 5 September 2016
Accepted 7 September 2016
Available online 9 September 2016

Keywords:
Lysosomal autophagy
Obesity and diabetes
Cardiomyopathy
Glucolipotoxicity
TFEB
LAMP-2A

ABSTRACT

Impaired cardiac metabolism in the obese and diabetic heart leads to glucolipotoxicity and ensuing cardiomyopathy. Glucolipotoxicity causes cardiomyocyte injury by increasing energy insufficiency, impairing proteasomal-mediated protein degradation and inducing apoptosis. Proteasome-evading proteins are degraded by autophagy in the lysosome, whose metabolism and function are regulated by master regulator transcription factor EB (TFEB). Limited studies have examined the impact of glucolipotoxicity on intra-lysosomal signaling proteins and their regulators. By utilizing a mouse model of diet-induced obesity, type-1 diabetes (Akita) and *ex-vivo* model of glucolipotoxicity (H9C2 cells and NRCM, neonatal rat cardiomyocyte), we examined whether glucolipotoxicity negatively targets TFEB and lysosomal proteins to dysregulate autophagy and cause cardiac injury. Despite differential effects of obesity and diabetes on LC3B-II, expression of proteins facilitating autophagosomal clearance such as TFEB, LAMP-2A, Hsc70 and Hsp90 were decreased in the obese and diabetic heart. *In-vivo* data was recapitulated in H9C2 and NRCM cells, which exhibited impaired autophagic flux and reduced TFEB content when exposed to a glucolipotoxic milieu. Notably, overloading myocytes with a saturated fatty acid (palmitate) but not an unsaturated fatty acid (oleate) depleted cellular TFEB and suppressed autophagy, suggesting a fatty acid specific regulation of TFEB and autophagy in the cardiomyocyte. The effect of glucolipotoxicity to reduce TFEB content was also confirmed in heart tissue from patients with Class-I obesity. Therefore, during glucolipotoxicity, suppression of lysosomal autophagy was associated with reduced lysosomal content, decreased cathepsin-B activity and diminished cellular TFEB content likely rendering myocytes susceptible to cardiac injury.

1. Introduction

A subset of obese and diabetic patients suffers from cardiac muscle specific contractile dysfunction termed as cardiomyopathy [1,2,3,4,5]. Etiology in the progression of cardiomyopathy in rodent models of obesity and diabetes includes diastolic dysfunction [6,7], myocyte hypertrophy [8,9,10], interstitial fibrosis [8,11,12], early-onset metabolic maladaptation [9,10,13,14], and progressive lipid accumulation [9,10], all of which precede heart failure. The underlying cause for metabolic inflexibility in cardiomyopathy is the lack of insulin or insulin action resulting in hyperglycemia and fatty acid overutilization, both of which lead to a condition known as "glucolipotoxicity" [1,15,16,17]. Numerous studies have demonstrated that glucolipotoxic effects in the cardiomyocytes, originate or terminate primarily in the mitochondria and the endoplasmic reticulum (ER) [18,19,20,21,22].

A glucolipotoxic milieu in the cardiomyocyte impairs protein quality control, inducing ER stress and activating protein degradation pathway [23,24]. To counter the damaging effects of glucolipotoxicity, proteasomal degradation is acutely activated to clear the cellular proteotoxic load [25]. Indeed, ubiquitin mRNA levels, caspase-3 and

Abbreviations: ATGL, Adipose triglyceride lipase; CHOP, CCAAT-enhancer-binding protein homologous protein; CLEAR, coordinated lysosomal enhancement and regulation; CMA, chaperone mediated autophagy; CQ, chloroquine; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; FA, fatty acids; GTT, glucose tolerance test; HFHS, high fat high sucrose; Hsc70, heat shock cognate protein 70; Hsp90, heat shock protein 90; ITT, insulin tolerance test; LAMP-2A, lysosome associated membrane protein 2A; LC3B, microtubule associated protein light chain 3 subtype B; MTCO1, mitochondrially encoded cytochrome C oxidase I; mTOR, mammalian target of rapamycin; NRCM, neonatal rat cardiomyocytes; STZ, streptozotocin; TFEB, transcription factor EB.

* Corresponding author.
E-mail address: tpulinil@dal.ca (T. Pulinilkunnil).

http://dx.doi.org/10.1016/j.bbalip.2016.09.004
1388-1981/

ATP-dependent proteasomal degradation are augmented acutely following streptozotocin (STZ)-induced type-1 diabetes [25]. However, sustained glucolipotoxicity exacerbates ER stress, saturating and impairing proteasomal protein degradation, causing toxic accumulation of misfolded proteins [26,27]. In agreement with this theory, failing hearts from chronically obese humans with type-2 diabetes display significant accumulation of non-degraded proteins [28], suggesting that impaired proteasomal degradation in late stages of obesity and diabetes promotes a maladaptive buildup of cytotoxic proteins causing and/or exacerbating cardiomyopathy. Recent experimental evidence suggests that if the proteasome is impaired then damaged proteins must be degraded by the lysosomal machinery via autophagy to maintain cellular homeostasis [29,30,31]. Interestingly, islet dysfunction in *ob*/*ob* mice is exacerbated following treatment with lysosomal function inhibitors [32], suggesting that disruption in lysosomal function accelerates cell death. However, the underlying mechanisms by which glucolipotoxicity affects inter- and intra-lysosomal signaling, metabolism and function remain to be examined. Furthermore, whether clinically observed proteotoxicity and cardiomyopathy in obese and diabetic hearts involves negative targeting of lysosomes by glucolipotoxic substrates remains to be investigated.

Autophagy degrades short- and long-lived proteins in the lysosome [33,34,35] either via macroautophagy [36,37,38] or via chaperone-mediated autophagy (CMA) [39,40]. Macroautophagy in the ER-cytosol interface requires lipidation of microtubule-associated protein 1 light chain B subtype 3 (LC3B-I) to form LC3B-II [37,41] resulting in autophagosome formation, maturation and fusion with the lysosome to degrade proteins. The macroautophagy process utilizes polyubiquitin cargo-receptor, p62/SQSTM1 to engage in partial-selection of bulk load of intracellular protein and organelle content [23,24] and therefore, changes in LC3B and p62/SQSTM1 signify changes in macroautophagy. Lysosomal CMA is a process by which cytosolic proteins targeted for degradation are delivered to lysosomal membrane protein-type 2A (LAMP-2A), which internalizes the protein cargo for lysosomal degradation [39,40]. Notably, humans and mice with loss of function of LAMP-2 exhibit impaired autophagosome clearance, lysosomal dysfunction, and cardiomyopathy, suggesting that lysosomal autophagy is critical for cardiac function [42,43,44]. Expression of numerous lysosomal proteins responsible for autophagic processes are under the direct control of transcription factor EB (TFEB), a transcriptional regulator of lysosome autophagy and biogenesis [45,46]. TFEB-action not only generates autophagosomes, but also accelerates their delivery and clearance by lysosomes via increases in lysosomal biogenesis [46]. It is plausible that changes in lysosome function and biogenesis could significantly impact cellular function in the setting of obesity, insulin resistance and diabetes, however, limited studies have examined the impact of glucolipotoxicity on TFEB and its downstream functions.

In this study, we examined whether glucolipotoxicity negatively targets TFEB, a transcriptional regulator of lysosome function, to impair autophagy and thereby render cardiomyocytes susceptible to proteotoxicity and cell death. Utilizing a mouse model of diet-induced obesity, type-1 diabetes and *ex-vivo* model of glucolipotoxicity (rat cardiomyofibroblasts and neonatal rat cardiomyocyte), we demonstrated that (1) baseline macroautophagy is reciprocally regulated in obesity and type-1 diabetes, (2) an obese and diabetic environment *in-vivo* suppressed lysosome signaling proteins, inhibited autophagic flux and reduced lysosomal proteolysis, (3) lysosomal protein suppression following *ex-vivo* myocyte nutrient overload is FA specific since palmitate or glucose/palmitate but not oleate or high glucose alone, reduces lysosomal protein content, (4) in the obese and diabetic heart, TFEB is decreased and this effect is recapitulated not only *ex-vivo* myocytes exposed to glucolipotoxic milieu but also in human heart tissue from patients with Class-1 obesity. Collectively, our data highlights a novel mechanism by which glucolipotoxicity targets TFEB to inhibit lysosomal integrity and render cardiomyocytes susceptible to proteotoxicity, injury and failure.

2. Experimental methods

2.1. Animal models

All protocols involving rodents were approved by the Dalhousie University, Institutional Animal Care and Use Committee.

2.1.1. Diet induced obesity

Male C57BL/6J mice were procured from the Jackson laboratory (Stock number; 000664). Mice were housed on a 12 h light and 12 h dark cycle with ad libitum access to water and chow diet (5001; Lab diet, St Louis, MO, USA; with 13.5 kcal% from fat) or high fat-high sucrose (HFHS) diet (12451; Research Diets, New Brunswick, NJ, USA; with 45 kcal% from fat and 17 kcal% from sucrose). Nine to ten weeks old male mice were randomly assigned to cohorts fed either chow or HFHS diet for 16 weeks. Body weight gain was recorded weekly by taking the differences in body weight before starting the diet and every week after starting the diet. The weight gain was recorded every week for 16 weeks. After 16 weeks, mice were injected with either saline or chloroquine (CQ, C6628; Sigma) for the last 48 h (30 mg/kg), 24 h (30 mg/kg) and 2 h (50 mg/kg) prior to euthanasia to assess autophagic flux [47]. All the mice were subjected to 1 h food withdrawal before euthanasia. Tissues were collected and stored at −80 °C for further analysis.

2.1.2. Akita mice

Male C57BL/6J wild-type (WT; *Ins2*$^{WT/WT}$) and type-1 diabetic Akita (*Ins2*$^{WT/C96Y}$) mice were purchased from Jackson Laboratory (stock numbers: WT, 000664; *Ins2*$^{WT/C96Y}$, 003548). Mice were housed on a 12 h light and12 h dark cycle with ad libitum access to chow diet (5001; Lab diet, St Louis, MO, USA; with 13.5 kcal% from fat) and water. Body weight was recorded every week. After 12 weeks, mice were euthanized after 12 h of overnight fasting and tissues were collected and stored at −80 °C for further analysis.

2.2. Induction of diabetes using streptozotocin mice

Male C57BL6J mice were injected with a single intraperitoneal dose of streptozotocin (STZ; S0130, Sigma) in saline at 175 mg/kg body weight. After 4 weeks, mice were euthanized in a fed state and tissues were collected and stored at −80 °C for further analysis.

2.3. Glucose tolerance test

Glucose tolerance was assessed in 15 h fasted non-anesthetized mice following intraperitoneal (i.p.) administration of 20% D-glucose in saline at 2 g/kg of body weight. Blood glucose was measured at 0, 15, 30, 60 and 120 min after glucose administration using Accu-Check Aviva glucometers (Roche, Basel, CH).

2.4. Insulin tolerance test

Systemic insulin tolerance was assessed in 5 h fasted non-anesthetized mice following intraperitoneal (i.p.) administration of human insulin (Novo Nordisk, Bagsvaerd, DK) at 0.8 units/kg body weight. Blood glucose was measured at 0, 15, 30, 60, 90 and 120 min after insulin administration using Accu-Check Aviva glucometers.

2.5. Tissue homogenization

Frozen hearts were powdered and homogenized in ice-cold lysis buffer [containing 20 mM Tris–HCl, pH 7.4, 5 mM EDTA, 10 mM Na_4P_2O7 (567540; Calbiochem, NJ, USA), 100 mM NaF, 1% Nonidet P-40, 2 mM Na_3VO_4, protease inhibitor (P8340, 10 μl/ml; Sigma, MO, USA) and phosphatase inhibitor (524628, 10 μl/ml, Calbiochem, NJ, USA)]. Homogenate was centrifuged at 1000*g* for 10 min at 4 °C to