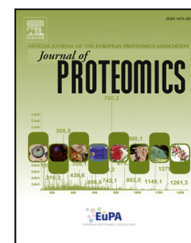
JOURNAL OF PROTEOMICS XX (2014) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



1 # Targets of protein carbonylation in spontaneously
2 hypertensive obese Koletsky rats and healthy
3 Wistar counterparts: A potential role on
4 metabolic disorders

Q1 Lucía Méndez[a], Manuel Pazos[a,*], Montserrat Giralt[c], M. Rosa Nogués[c], Jara Pérez-Jiménez[b],
6 Josep L. Torres[b], J.M. Gallardo[a], Isabel Medina[a]

7 [a]Instituto de Investigaciones Marinas (IIM-CSIC), Eduardo Cabello 6, E-36208 Vigo, Spain
8 [b]Instituto de Química Avanzada de Catalunya (IQAC-CSIC), Jordi Girona 18-26, E-08034 Barcelona, Spain
9 [c]Unidad de Farmacología, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili, Sant Llorenç 21, E-43201 Reus, Spain
10

13 A R T I C L E I N F O

14 Article history:
15 Received 20 February 2014
16 Accepted 20 April 2014
17

43 Keywords:
44 Metabolic syndrome
45 SHROB model
46 Wistar rat
47 Oxidative stress
48 Redox proteomics
49 Protein carbonylation
50

A B S T R A C T

The study innovatively pinpoints target proteins of carbonylation, a key PTM induced by oxidative stress, in the SHROB (genetically obese spontaneously hypertensive) rat model of metabolic syndrome (MetS). Protein carbonylation was assessed by a fluorescence-labeling proteomics approach, and complemented with biometric and biochemical markers of MetS. SHROB and healthy Wistar rats were fed two diets, soybean and linseed oil supplementations, in order to distinguish intrinsic carbonylation of SHROB animals from diet-modulated carbonylation unrelated to MetS. First exploratory data showed similar carbonylation patterns and metabolic conditions in SHROB rats fed soybean and linseed, but different from Wistar animals. A total of 18 carbonylated spots in liver, and 12 in skeletal tissue, related to pathways of lipid (29.6%), carbohydrate (25.9%) and amino acid (18.5%) metabolisms, were identified. In particular, SHROB animals present higher carbonylation in four liver proteins belonging to lipid metabolism, redox regulation and chaperone activity (ALDH2, PDI, PDIA3, PECR), and in the skeletal muscle ALDOA is involved in muscle dysfunction. Conversely, SHROB rats display lower carbonylation in liver albumin, AKR1C9, ADH1 and catalase. This investigation provides a novel perspective of carbonylation in the context of metabolic disorders, and may be a starting point to characterize new redox pathways exacerbating MetS. 18–33 Q3

34

35 Biological significance

Oxidative stress is a concomitant factor in the pathogenesis of MetS that induces oxidative PTM as carbonylation. Through the use of a redox proteomics approach, we have thoroughly mapped the occurrence of protein targets of carbonylation in the genetically-induced MetS model SHROB rat. The present research brings a new insight of MetS pathogenesis and it may provide valuable information to understand the biological impact of oxidative stress in patients with MetS. 36–41

© 2014 Published by Elsevier B.V. 42

53
54

* Corresponding author. Tel.: +34 986 231930; fax: +34 986 292762.
E-mail address: mpazos@iim.csic.es (M. Pazos).

http://dx.doi.org/10.1016/j.jprot.2014.04.036
1874-3919/© 2014 Published by Elsevier B.V.

UNCORRECTED PROOF

ARTICLE IN PRESS

1. Introduction

The combination of obesity, dyslipidemia, hypertension and insulin resistance, known as metabolic syndrome (MetS), is a potential risk factor for type-2 diabetes and cardiovascular diseases [1]. Several pieces of evidence indicate a key role of oxidative stress, a condition in which an imbalance results between production and inactivation of reactive oxygen species (ROS), in the pathogenesis of MetS. Obesity and hypertriglyceridemia were positively correlated with systemic oxidative stress in humans and mice [2,3]. ROS generation, accompanied by increased expression of NADPH oxidase and reduced expression of antioxidant enzymes, was enhanced in adipose tissue of obese mice, whereas the reduction of ROS by treatment with NADPH oxidase inhibitor diminished ROS production and improved diabetes, hyperlipidemia, and hepatic steatosis [4]. Monocytes enhance superoxide radical production under hypertriglyceridemia stimuli [5]. Oxidative stress is also present in subjects with either pre-diabetes or diabetes [6], and high levels of glucose stimulate mitochondrial superoxide radical output [7]. In primate models, hyperglycemia was able to promote oxidation of artery wall proteins via hydroxyl radical-like species [8]. Although overproduction of ROS causes biological damage, low/moderate ROS concentrations are required in normal physiological conditions due to ROS function in a number of cellular signaling pathways and in the induction of a mitogenic response [9].

Oxidative stress may trigger cell damage through deleterious oxidative modifications of essential biomolecules. In particular, proteins are major targets of ROS as a result of their abundance in biological systems and their elevated rate constants for reaction with radicals and non-radical oxidants [10]. Among the numerous types of PTM induced by oxidative stress, much of the attention has been focused on protein carbonylation [11]. Protein carbonylation is more hardly induced compared with methionine sulphoxidation and cysteinyl oxidative derivatives, and might therefore be indicative of a more severe oxidative environment [12]. In fact, elevated levels of protein carbonyls are generally interrelated not only with oxidative stress episodes but also with disease-derived protein dysfunction. Several evidences indicate a straightforward relationship between protein carbonylation and MetS-associated disorders. Patients with type-2 diabetes have an increased concentration of plasma protein carbonyls compared to healthy controls, but no significant differences in plasma thiol concentration [13]. Rodents fed diets inducing MetS disorders, high-fat and/or high sucrose, have shown higher global carbonylation in brain [14] and liver [15], and also increased carbonylation of a number of adipose-regulatory proteins [16], suggesting protein carbonylation as a mechanistic link between oxidative stress and MetS. Accordingly, the treatment of carbonyl stress by redox modulation, protein carbonyl detoxification and inhibition of carbonyl generation offers new therapeutic approaches in diabetes and arteriosclerosis [12].

The Koletsky strain of rats, a genetically obese spontaneously hypertensive rat (SHROB), is a well-accepted animal model for understanding metabolic abnormalities associated with MetS [17]. SHROB obese genotype contains a mutation in the leptin receptor gene that causes resistance to leptin, an adipose hormone that acts via hypothalamus reducing appetite and regulating metabolism. SHROB strain displays an increased oxidative stress [18], together with metabolic disorders implicated in the clustering of abnormalities of MetS, such as obesity, hypertension, insulin resistance, hyperglucagonemia and hypertriglyceridemia [17]. However, it is noteworthy the lack of information about which are the major target proteins of carbonylation in the SHROB model, despite the robust evidence that links MetS and oxidative stress, and the potential impact that protein carbonylation may have on enzymatic activity and the development of metabolic disorders.

The present investigation was addressed to comprehensively map protein carbonyls in SHROB animals genetically predisposed to develop MetS. Wistar rats were used as healthy control animals. Animals were fed two diets, soybean and linseed oil supplementations, with the aim of identifying carbonylation targets inherent of the SHROB model rather than diet-modulated carbonylation unrelated to MetS. Soybean oil is a rich source of the n−6 linoleic acid (LA, 18:2) and a principal source of fat in standard murine chows; meanwhile, linseed oil is an important dietary source of the n−3 alpha-linolenic (ALA, 18:3), a component with potential preventive effects on metabolic alterations such as glucose tolerance, insulin sensitivity, dyslipidemia and hypertension [19]. Protein targets of carbonylation, and the extent of such PTM, were evaluated by a redox proteomics approach that combined fluorescent chemical-tagging with the carbonyl-specific fluorescein-5-thiosemicarbazide (FTSC), imaging analysis on gel electrophoresis and tandem mass spectrometry.

2. Material and methods

2.1. Material and reagents

Pure organic soybean oil first cold pressed was obtained from Clearspring Ltd. (London, UK), and pure linseed oil first cold pressed was purchased from Biolasi S.L. (Ordizia, Guipuzcoa, Spain). Ketamine chlorhydrate (Imalgene 1000) was purchased from Merial Laboratorios (Barcelona, Spain) and xylacine (Rompun 2%) was from Quimica Farmaceutica (Barcelona, Spain). ProteoBlock™ protease inhibitor cocktail was purchased from Thermo Fisher Scientific (Rockford, USA). Tris–HCl, phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), iodoacetamide, ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), 3,3-cholaminopropyl-dimethylammonio-1-propanesulfonate (CHAPS), sodium phosphate, magnesium chloride anhydrous and bicinchoninic acid (BCA) were purchased from Sigma (St. Louis, MO). Urea, thiourea, sodium dodecyl sulfate (SDS), glycine, glycerol and Serdolit MB-1 were obtained from USB (Cleveland, OH). Immobiline™ DryStrip gels (pH 3–10, 11 cm), IPG buffer, pharmalyte 3–10, ammonium persulfate (APS), bromophenol blue, and 1,2-bis(dimethylamino)-ethane (TEMED) were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Acrylamide, bis N,N′-methylene-bis-acrylamide and Bio-Rad protein assay were obtained from Bio-Rad (Hercules, CA). Fluorescein-5-thiosemicarbazide (FTSC) was obtained from Invitrogen (Carlsbad, CA) and porcine sequencing grade modified trypsin was from Promega (Madison, WI). All other chemicals and reagents used were of analytical reagent grade and water was purified using a Milli-Q system (Millipore, Billerica, MA).

UNCORRECTED PROOF

Please cite this article as: Méndez L, et al, Targets of protein carbonylation in spontaneously hypertensive obese Koletsky rats and healthy Wistar counterparts: A potential role..., J Prot (2014), http://dx.doi.org/10.1016/j.jprot.2014.04.036

Download English Version:

https://daneshyari.com/en/article/7636303

Download Persian Version:

https://daneshyari.com/article/7636303

Daneshyari.com