The International Journal of Biochemistry & Cell Biology 72 (2016) 18–26

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Leptin regulates energy metabolism in MCF-7 breast cancer cells

Mª del Mar Blanquer-Rosselló[a,b,c], Jordi Oliver[a,b,c], Jorge Sastre-Serra[a,b,c], Adamo Valle[a,b,c,*], Pilar Roca[a,b,c]

[a] Grupo Multidisciplinar de Oncología Traslacional, Institut Universitari díInvestigació en Ciències de la Salut (IUNICS), Palma de Mallorca, Illes Balears, Spain
[b] Ciber Fisiopatología Obesidad y Nutrición (CB06/03) Instituto Salud Carlos III, Madrid, Spain
[c] Instituto de Investigación Sanitaria de Palma (IdISPa), Hospital Universitario Son Espases, edificio S., E-07120 Palma de Mallorca, Illes Balears, Spain

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
14 December 2015
Accepted 5 January 2016
Available online 6 January 2016

Keywords:
Breast cancer
Leptin
Warburg effect
Metabolism
Lipid oxidation
Glycolysis

ABSTRACT

Obesity is known to be a poorer prognosis factor for breast cancer in postmenopausal women. Among the diverse endocrine factors associated to obesity, leptin has received special attention since it promotes breast cancer cell growth and invasiveness, processes which force cells to adapt their metabolism to satisfy the increased demands of energy and biosynthetic intermediates. Taking this into account, our aim was to explore the effects of leptin in the metabolism of MCF-7 breast cancer cells. Polarographic analysis revealed that leptin increased oxygen consumption rate and cellular ATP levels were more dependent on mitochondrial oxidative metabolism in leptin-treated cells compared to the more glycolytic control cells. Experiments with selective inhibitors of glycolysis (2-DG), fatty acid oxidation (etomoxir) or aminoacid deprivation showed that ATP levels were more reliant on fatty acid oxidation. In agreement, levels of key proteins involved in lipid catabolism (FAT/CD36, CPT1, PPARα) and phosphorylation of the energy sensor AMPK were increased by leptin. Regarding glucose, cellular uptake was not affected by leptin, but lactate release was deeply repressed. Analysis of pyruvate dehydrogenase (PDH), lactate dehydrogenase (LDH) and pyruvate carboxylase (PC) together with the pentose-phosphate pathway enzyme glucose-6 phoshate dehydrogenase (G6PDH) revealed that leptin favors the use of glucose for biosynthesis. These results point towards a role of leptin in metabolic reprogramming, consisting of an enhanced use of glucose for biosynthesis and lipids for energy production. This metabolic adaptations induced by leptin may provide benefits for MCF-7 growth and give support to the reverse Warburg effect described in breast cancer.

1. Introduction

Altered tumor metabolism is one of the hallmarks of cancer. The pioneer work of Warburg and colleagues in 1920s showed that cancer cells metabolize glucose to lactate despite oxygen presence, phenomenon known as the Warburg effect or aerobic glycolysis (Koppenol et al., 2011). Recently it has been demonstrated that cancer cell metabolism goes beyond a simple interplay between glycolysis and mitochondria, given that the anabolic pathways are necessary to provide proteins, fatty acids and nucleic acids for rapid tumor proliferation (Vander Heiden et al., 2009). Thus, cancer cells need the glycolytic and pentose phosphate pathway as well as the Krebs cycle for their biosynthetic intermediates. In spite of Warburg's first assumption that mitochondrial respiration is impaired in tumor cells, recent findings evidence the presence of functional mitochondria in these cells. Indeed, some tumors either rely mainly on oxidative phosphorylation (Moreno-Sanchez et al., 2007; Ward and Thompson, 2012; Whitaker-Menezes et al., 2011a) or switch between aerobic glycolysis and oxidative metabolism depending on their microenvironment, thus giving them metabolic flexibility (Rodriguez-Enriquez et al., 2001). Recently, Lisanti and colleagues have described for human breast cancer that aerobic glycolysis actually takes place in tumor-associated fibroblasts, and not properly in the epithelial cancer cells (Pavlides et al., 2009). This innovative idea, termed as "The Reverse Warburg effect", proposes

Abbreviations: 2-DG, 2-deoxyglucose; AMPK, 5′ AMP-activated protein kinase; FAT/CD36, fatty acid translocase or cluster of differentiation 36; CPT1, carnitine palmitoyltransferase I; CS, citrate synthase; G6PDH, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT2, glucose transporter 2; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; pAMPK, phosphorylated 5′ AMP-activated protein kinase; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PPARα, peroxisome proliferator-activated receptor alpha.

* Corresponding author at: Departamento de Biología Fundamental y Ciencias de la Salud, Universitat de les Illes Balears. Ed Guillem Colom Cas. Cra Valldemossa Km 7.5, 07122 Palma de Mallorca, Spain. Tel.: +34 971259644; fax: +34 971 173184.
E-mail address: adamo.valle@uib.es (A. Valle).

http://dx.doi.org/10.1016/j.biocel.2016.01.002
1357-2725/

that aerobic glycolysis in cancer-associated fibroblasts results in the production of high-energy metabolites (such as lactate and pyruvate), which can then be transferred to adjacent epithelial cancer cells undergoing oxidative mitochondrial metabolism (Pavlides et al., 2009; Bonuccelli et al., 2010; Whitaker-Menezes et al., 2011b).

Obesity greatly influences risk, prognosis and progression of certain types of cancer (Wolin et al., 2010; Majed et al., 2008). The prevalence of breast cancer in postmenopausal obese women has been attributed to altered endocrine status, with estrogen and leptin being the most prominent involved factors (Calle and Thun, 2004; Cleary and Maihle, 1997; Willett, 1997; Porter et al., 2006). In fact, various studies have shown that leptin influences almost all stages of tumorigenesis acting in an endocrine, paracrine and autocrine manner (Saxena and Sharma, 2013). Leptin is a peptide hormone principally secreted by adipocytes and to a lesser extent by other tissues (Saxena and Sharma, 2013; Muoio and Lynis Dohm, 2002). Serum leptin levels rise with increasing adiposity (Muoio and Lynis Dohm, 2002) and regulate energy intake and expenditure through its receptor located in the neurons of the hypothalamic nuclei (Schwartz et al., 1996). However, the leptin receptor (OB-R) has a widespread expression in peripheral tissues including liver, pancreas, adipose tissue, skeletal muscle and mammary gland (Mercer et al., 1996; Ahima and Flier, 2000; Schubring et al., 1999). This ubiquitous OB-R distribution in almost all tissues explains the underlying pleiotropic roles of leptin (Fruhbeck, 2006; Anubhuti and Sarika Arora, 2008).

A hyperactive leptin-signaling network leads to the concurrent activation of multiple pathways involved in proliferation, resistance to apoptosis, cell adhesion, invasion and migration in breast cancer cells (Saxena and Sharma, 2013; Saxena et al., 2007; Somasundar et al., 2004). Nevertheless, little is known about the effects of leptin in breast cancer metabolism. In other tissues such as skeletal muscle and fat, receptor-leptin binding promotes energy dissipation and prevents fatty acid accumulation by increasing fatty acid oxidation (Ceddia, 2005). Several reports suggest that one of the targets of leptin action in these tissues is the energy sensing 5′-AMP-activated protein kinase (AMPK) which plays a major role in the regulation of cellular lipid and protein metabolism (Minokoshi et al., 2002; Janovska et al., 2008; Steinberg et al., 2003).

Given the ability of leptin to influence cellular metabolism and taking into consideration the importance of energy production and the biosynthetic processes necessary to support rapid cell growth, a more comprehensive assessment of the influence of this adipokine on the metabolic features of breast epithelial cancer cells may shed new light on the association between obesity and a poor prognosis for breast cancer. Thus, the aim of this work is to better understand the role of leptin on MCF-7 breast cancer cells metabolism to further elucidate how this adipokine participates in the induction of the proliferative response in cancer cells.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM) high glucose without phenol red was from GIBCO (Paisley, UK). Charcoal-stripped fetal bovine serum (FBS) and penicillin-streptomycin were from Biological Industries (Kibbutz Beit Haemek, Israel). 2-DG, oligomycin, etomoxir and leptin were from Sigma-Aldrich (St. Louis, MO). ApoSensor™ Cell Viability Assay Kit was purchased from BioVision (Milpitas, CA) and BCA protein assay from Pierce (Bonn, Germany). The Glucose MR Kit was from Bioquimica linear Chemicals/Cromatest (Barcelona, Spain). Total OXPHOS human WB antibody cocktail (#MS601) was from MitoSciences (Eugene, OR, USA). Human WB antibodies AMPKα (SC-25792), phospho-AMPKα (SC-33524), FAT/CD36 (SC-9154), CPT1 (SC-20669), GAPDH (SC-25778), PDH-E1 (SC-377092), PPARα (SC-9000) and tubulin (SC-5286) were from Santa Cruz Biotechnology (Texas, USA). Human WB antibody GLUT2 (AB-1342) was from Chemicon International (Temecula, CA, USA) and LDH (#2012) was from Cell Signaling (Danvers, MA, USA). Immun-Star© Western C© Chemiluminescent Kit was from Bio Rad Laboratories (Hercules, CA). Finally, PDH Enzyme Activity Microplate Assay Kit was from Abcam (Cambridge, UK).

2.2. Cell culture

MCF-7 breast cancer cell line was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in DMEM high glucose without phenol red supplemented with 10% charcoal-stripped FBS and 1% antibiotic (penicillin and streptomycin) at 37 °C in 5% CO_2. Cell were treated when cultures reached 80% confluence by providing fresh medium supplemented with 100 ng/mL leptin or without (control cells) for 24 h.

2.3. Oxygen consumption rate

After leptin treatment, cells were harvested and 10^6 cells were incubated in 0.5 mL of DMEM high glucose without phenol red supplemented with 10% charcoal-stripped FBS in a water-thermostatically regulated chamber with a computer-controlled Clark-type O_2 electrode (Oxygraph; Hansatech, Norfolk, UK). Cells were preincubated for 5 min at 37 °C and basal respiration rate was measured three times during the following 5 min. Next, sequential aliquots of 2-DG were added in order to obtain concentrations of 5, 10 and 20 mM into the chamber and respiration rate was measured. The same procedure was followed for oligomycin (0.25, 0.5 and 1 μM).

2.4. Intracellular ATP levels

Cells were seed in 96-well plates and cultured for 24 h with leptin. ATP levels were measured using the ApoSensor™ Cell Viability Assay Kit, after treatment with different concentrations of 2-DG or oligomycin for 30 min. For assessment of fatty acid oxidation cells were preincubated with 50 μM etomoxir for 30 min prior to 2-DG addition. For amino acid deprivation experiment, media was shifted to Krebs-Ringer modified buffer (NaCl 145 mM, KCl 4.86 mM, $CaCl_2$ 0.54 mM, $MgSO_4$ 1.22 mM, 5.7 mM NaH_2PO_4, glucose 4.5 g/L and 10% charcoal-stripped FBS) 30 min before 20 mM 2-DG addition. For data normalization, cell number was determined in parallel treated wells by the Crystal Violet method (Nagamine et al., 2009). Briefly, 20 μL of violet crystal solution (0.5% of violet crystal in 30% acetic acid) were added to each well and incubated for 10 min at room temperature. The plate was washed twice with distilled water and dye solubilized with 100 μl of methanol shaking for 1 min. Finally, absorbance was measured at 570 nm using a microplate reader (Power Wave XS, Bio-Tek).

2.5. Western blot

MCF-7 cells were treated with leptin for 24 h and lysates were prepared by scraping cells in RIPA buffer (50 mM Tris–HCl, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% Triton x-100, 1 mM EDTA, 1 mM Na_3VO_4, 2 m PMSF, 0.01 mM leupeptin, 0.01 mM pepstatin and 1 mM NaF; pH 7.5) and disrupted by sonication. Protein content was determined with a BCA protein assay kit. For Western blot analysis, 35 μg of protein from cell lysates were separated on SDS–PAGE gels and electrotransferred onto nitrocellulose membranes. Membranes were incubated in a blocking solution of 5%