FEBS Letters 589 (2015) 608–614

journal homepage: www.FEBSLetters.org

Dynamic subcellular localization of aquaporin-7 in white adipocytes

Takayuki Miyauchi [a,*,1], Hiroyuki Yamamoto [a,1], Yoichiro Abe [a], Go J. Yoshida [a], Aleksandra Rojek [b], Eisei Sohara [c], Shinichi Uchida [c], Søren Nielsen [b], Masato Yasui [a,*]

[a] Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
[b] Department of Health Science and Technology, Aalborg University, Fredrik Bajersvej 7, 9220 Aalborg, Denmark
[c] Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima, Bunkyo, Tokyo 113-8519, Japan

ARTICLE INFO

Article history:
Received 8 October 2014
Revised 15 December 2014
Accepted 15 January 2015
Available online 30 January 2015

Edited by Laszlo Nagy

Keywords:
Lipolysis
Adipocyte
Aquaporin-7
Comparative gene identification 58

ABSTRACT

Aquaporin-7 (AQP7) is expressed in adipose tissue, permeated by water and glycerol, and is involved in lipid metabolism. AQP7-null mice develop obesity, insulin resistance, and adipocyte hypertrophy. Here, we show that AQP7 is expressed in adipocyte plasma membranes, and is re-localized to intracellular membranes in response to catecholamine in mouse white adipose tissue. We found that internalization of AQP7 was induced by PKA activation and comparative gene identification 58 (CGI-58). This relocation was confirmed by functional studies in 3T3-L1 adipocytes. Collectively, these results suggest that AQP7 makes several contributions to adipocyte metabolism, in both cortical and intracellular membranes.

1. Introduction

White adipocytes play an indispensable role in controlling whole body metabolism via their management of lipid storage, as well as the production of bioactive peptides and the regulation of insulin sensitivity [1,2]. In adipocytes, lipids are mainly stored as triacylglycerol (TG) in lipid droplets (LDs). Recent studies revealed that the surfaces of LDs and LD-associated endoplasmic reticulum (ER) are platforms where various proteins are associated and engaged in lipolysis and lipogenesis [3–5]. For example, the association of adipose triglyceride lipase (ATGL) with comparative gene identification 58 (CGI-58) at the LD surface is the initiation step in TG catabolism [6]. The glycerol-3-phosphate acyltransferase GPAT4, which catalyzes the rate-limiting step in TG synthesis, functions at the ER and forming LDs [7,8]. The subcellular distribution of glycerol and the mechanism controlling its local concentration in lipolytic or lipogenetic adipocytes remain unclear.

Aquaporin-7 (AQP7) is an aquaglyceroporin expressed in adipose tissues [9–11]. Dysregulation of glycerol concentration is a remarkable contributing factor to the development of metabolic disease. This is supported by the observation that AQP7-null mice exhibit marked hypertrophy of adipocytes, obesity and diabetes [12,13]. Furthermore, the activity of glycerol kinase, which is normally suppressed in adipocytes, is augmented in AQP7-null mouse adipocytes, accompanied by the accumulation of glycerol and TG and hypertrophy [12]. The involvement of AQP7 in adipocyte lipid metabolism is also supported by the findings that AQP7 binds LDs in 3T3-L1 adipocytes [14] and that AQP7 expression improves adipose tissue insulin resistance [15]. However, the extent and the subcellular localization of AQP7 in adipose tissue remain controversial [16]: (i) We and others reported the phenotype of AQP7-null mice, which displays the metabolic disorder in adipocytes [12,13]; and (ii) another study [17] demonstrated restricted AQP7 expression in the capillary endothelia of adipose tissue, but not in the surrounding adipocytes of mouse adipose tissue, suggesting a non-cell autonomous role for AQP7 in promoting antihypertrophic effects.

In this study, we first aimed to investigate the reasons for the discrepancies in the expression profiles where adipocyte metabolism was affected by AQP7 in nearby capillary endothelia. Our results demonstrated that AQP7 is expressed in mouse white adipocytes, and that AQP7 translocates from cortical to intracellular membranes during lipolysis. We identified the signaling mechanism controlling this translocation of AQP7.

Abbreviations: LD, lipid droplet; ER, endoplasmic reticulum; TG, triacylglycerol; PKA, protein kinase A; CARS, coherent anti-Stokes Raman scattering

* Corresponding authors. Fax: +81 3 3359 8889.
E-mail addresses: miyauchi@z3.keio.jp (T. Miyauchi), myasui@a3.keio.jp (M. Yasui).

[1] Miyauchi and Yamamoto contributed equally to this work.

http://dx.doi.org/10.1016/j.febslet.2015.01.025

2. Materials and methods

2.1. Mouse adipose tissue

All animal work was conducted in accordance with relevant guidelines and regulations. The generation of AQP7-null mice has been described previously [18]. White adipose tissues isolated from inguinal tissues of AQP7+/+ and AQP7-null C57BL/6 mice were cut into small pieces (less than 2 mm thick) and used for immunofluorescence imaging. To analyze AQP7 translocation, the small pieces of tissue were exposed to media containing 1 μm insulin or 10 μm norepinephrine and incubated at 37 °C for 3 h with shaking for aeration. Prior to fixation, the adipocytes in the tissue were confirmed to be at least 95% viable by calcein-AM incorporation.

2.2. Cell culture and differentiation

3T3-L1 cells were differentiated into adipocytes as described by Jain et al. [19]. The cells were transfected with expression plasmids by electroporation on 8 days after the induction of differentiation, incubated for 2 days, and then analyzed by microscopic imaging.

2.3. Immunohistochemistry and immunocytochemistry

Tissues and 3T3-L1 adipocytes were fixed using 10% trichloroacetic acid (TCA) as described by Hayashi et al. [20]. The affinity-purified anti-mouse AQP7 antibody (1246) has been previously characterized [21].

2.4. Imaging

During live imaging of lipolysis, the localization of AQP7-EGFP was observed with simultaneous monitoring of lipids using coherent anti-Stokes Raman scattering (CARS) microscopy [22]. Lipolysis was stimulated by bath- applications of 5 μm forskolin or 10 μm norepinephrine prepared in HEPES-buffered DMEM-F12 (Life technologies) medium containing 2% fetal bovine serum at 37 °C. The images were analyzed using FluoView software (ver. 2.01; Olympus), and Metamorph software (ver. 7.7; Molecular devices, Downingtown, PA).

Full methods and associated references are available in SI Materials and methods.

3. Results

3.1. Optimized staining of adipocytes with an anti-AQP7 antibody

Immunofluorescence imaging was optimized to detect a low level of AQP7 expression. TCA-fixation can improve the detection sensitivity similar to cases of ezrin/radixin/moesin or RhoA labeling [20,23]. First, we checked the mRNA expression of aqp7 in adipocyte differentiation-induced 3T3-L1 cells. The expression was observed specifically in 3T3-L1 adipocytes (Fig. 1A). Then, the specificity of the anti-AQP7 antibody in TCA-fixed samples was confirmed by a cell assay, in addition to a specificity test that we have performed in a previous study of paraformaldehyde (PFA)-fixed samples [21]. Intracellular flow cytometry analysis showed a

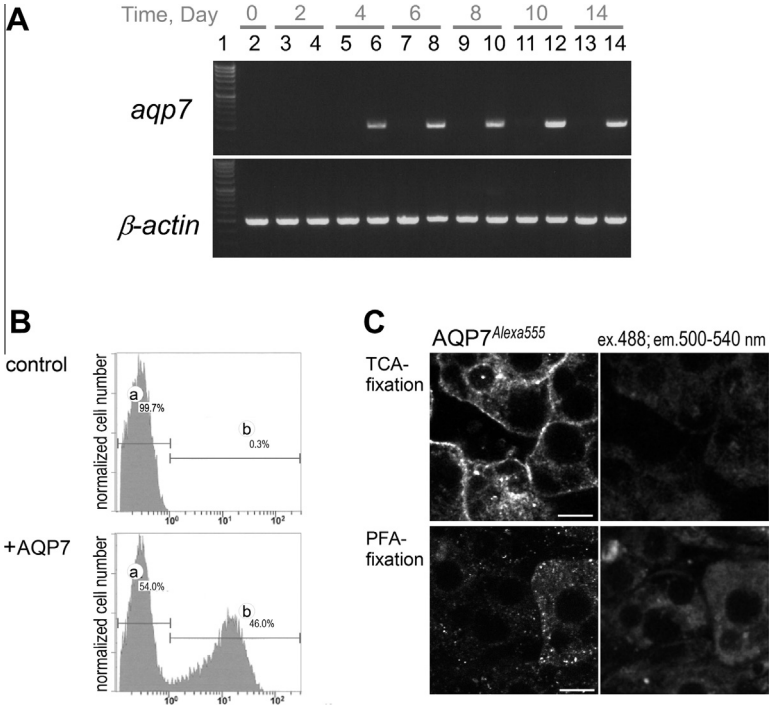

Fig. 1. Optimization of the immunostaining method to visualize the intracellular localization of AQP7 in 3T3-L1 cells. (A) Expression of aqp7 mRNA in differentiation-induced 3T3-L1 cells. Reverse transcription-PCR for 3T3-L1 adipocytes/pre-adipocytes was performed using primers specific for aqp7 and for the control, β-actin (lane 1: molecular weight marker; lanes 2, 3, 5, 7, 9, 11, 13: control 3T3-L1 pre-adipocytes; lanes 4, 6, 8, 10, 12, 14: 3T3-L1 adipocytes). Day: days after induction of differentiation. (B) The specific recognition of aquaporin-7 (AQP7) protein by the anti-AQP7 antibody was confirmed using cells selectively expressing AQP7. The normalized histograms of intracellular flow cytometric analysis are shown. Top panel: control 3T3-L1 cells transfected and partially expressing mCherry, fixed with trichloroacetic acid (TCA), and immunostained with an anti-AQP7 primary antibody and an Alexa488-labeled secondary antibody. Bottom panel: 3T3-L1 cells transfected and partially expressing AQP7-mCherry, fixed with TCA, and immunostained with an anti-AQP7 primary antibody and an Alexa488-labeled secondary antibody. Note that the horizontal axis shows the fluorescence intensity (excitation 488 nm; emission 525 ± 15 nm) of each cell in a common logarithm; the single peak separated from the background signals arises in an AQP7-dependent manner. Both histograms include 15 000 cells. (C) Comparison of the immunoreactivity of the anti-AQP7 antibody in 3T3-L1 adipocytes fixed with TCA or paraformaldehyde (PFA). The immunoreactivity of the anti-AQP7 antibody (left panel) and autofluorescence (right panel) in 3T3-L1 adipocytes fixed with TCA or PFA were also compared. The cells were labeled with anti-AQP7 and Alexa555-conjugated anti-rabbit antibodies (left panel). Bars = 10 μm.