FEBS Letters 582 (2008) 1685–1692

Increased abundance of cytoplasmic and nuclear caveolin 1 in human diploid fibroblasts in H_2O_2-induced premature senescence and interplay with $p38\alpha^{MAPK}$

Aline Chrétien, Neil Piront, Edouard Delaive, Catherine Demazy, Noëlle Ninane, Olivier Toussaint*

Unit of Research on Cellular Biology, University of Namur (FUNDP), Rue de Bruxelles 61, B-5000 Namur, Belgium

Received 18 March 2008; accepted 15 April 2008

Available online 23 April 2008

Edited by Vladimir Skulachev

Abstract Treatment of IMR-90 human diploid fibroblasts with a sublethal concentration of H_2O_2 induces premature senescence. We investigated the protein abundance, subcellular localization and involvement of caveolin 1 in premature senescence. Caveolin 1 is a scaffolding protein able to concentrate and organize signaling molecules within the caveolae membrane domains. We report the first evidence of increased nuclear and cytoplasmic localization of caveolin 1 during establishment of H_2O_2-induced premature senescence. Moreover, we demonstrate that phosphorylation of caveolin 1 during treatment with H_2O_2 is dependent on p38α mitogen-activated protein kinase.
© 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: H_2O_2; siRNA; Cellular senescence; Caveolin 1; Fibroblasts; Stress-induced premature senescence

1. Introduction

Senescent human diploid fibroblasts (HDFs) obtained after serial subcultivation display typically enlarged cell shape, senescence associated β-galactosidase activity (SA β-gal), changes in the expression level of many genes and irreversible growth arrested [1–4]. HDFs treated with sublethal concentrations of hydrogen peroxide (H_2O_2) or *tert*-butylhydroperoxide (*t*-BHP) display markers of senescence within two or three days after the stress [5–10]. This is stress-induced premature senescence (SIPS) [11].

Caveolin 1 (Cav1) is the principal structural component of caveolae membrane domains and is a scaffolding protein able to concentrate and organize signaling molecules within the caveolae [12,13].

Both N- and C-terminal domains of Cav1 face the cytoplasm and are accessible for interactions with cytoplasmically oriented molecules [14]. Cav1 mediates the inhibition of Erk and PI3K proteins in NIH3T3 fibroblasts under cell detachment [15] or induces Akt activation in mesangial human cells in presence of H_2O_2 [16,17].

Overexpression of Cav1 induces premature cellular senescence in primary cultures of murine fibroblasts [18]. In NIH3T3 murine fibroblasts and in endothelial cells treated with H_2O_2, $p38^{MAPK}$ is involved in the phosphorylation of Cav1 [19,20].

We show herein that Cav1 abundance is increased in cytoplasm and nucleus of IMR-90 fetal lung HDFs in H_2O_2-induced premature senescence. We used small interfering RNA (siRNA) to down-regulate Cav1 in order to test whether Cav1 is involved in premature senescence. siRNA were used to down-regulate $p38\alpha^{MAPK}$ and to study its role in the phosphorylation of Cav1 observed during treatment with H_2O_2.

2. Materials and methods

2.1. Cell culture, stress and stimulation

IMR-90 human fetal lung diploid fibroblasts (HDFs) were grown in minimum essential medium (MEM) (Gibco, UK) supplemented with 10% fetal bovine serum (FBS) (Gibco). IMR-90 HDFs at about 50% of in vitro proliferative life span (27 population doublings) were seeded at a density of 24×10^3 cells/cm^2. One day later, cells were treated for 1 or 2 h with 150 μM of H_2O_2 (Merck, Germany) diluted in MEM + 10% FBS. A 2 h incubation with H_2O_2 represents a sublethal treatment [6,21–23]. Controls cells were incubated in culture medium alone. After treatment, IMR-90 HDFs were washed twice with cold phosphate buffer saline pH 7.4 (10 mM phosphate, 0.9% NaCl) (PBS) and incubated with fresh MEM + 10% FBS. The appearance of biomarkers of senescence was checked (decrease of the proliferative potential and increase in SA β-gal-staining) [6,21,22] from the 2nd to the 3rd day after treatment.

2.2. RT-real time PCR

RNA extraction, choice of the sequences of primers and RT-real time polymerase chain reaction (PCR) was performed as detailed in [22]. Sequences of the primers can be found in Table 1A. The abundance of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger ribonucleic acid (mRNA) was used as a reference for semi-quantification with the classical "$\Delta\Delta C_T$ method" [24].

2.3. Western blots

Extraction of total proteins was performed as detailed in [22]. Extraction of nuclear proteins: cells were washed with PBS, scraped in hypotonic lysis buffer (HEPES 10 mM, pH 7.9, KCl 10 mM, EDTA

*Corresponding author. Fax: +32 81724135.
E-mail address: olivier.toussaint@fundp.ac.be (O. Toussaint).

Abbreviations: FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDF, human diploid fibroblast; IgG, immunoglobulin G; MAPK, mitogen-activated kinase; MEM, minimum essential medium; mRNA, messenger ribonucleic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay; PBS, phosphate buffer saline; RT-PCR, reverse transcriptase-polymerase chain reaction; S.D., standard deviation; SA β-gal activity, senescence associated-β galactosidase activity; siRNA, small interfering RNA; TGF-β1, transforming growth factor-β1

0014-5793/$34.00 © 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2008.04.026

Table 1A
Sequences of the primers used in RT real-time PCR analysis

Names	Sequences	GenBank
Cav1		NM_001753
Forward	5′-CGCACACCAAGGAGATCGA-3′	
Reverse	5′-GTGTCCCTTCTGGTTCTGCAAT-3′	
GAPDH		NM_002046
Forward	5′-ACCCACTCCTCCACCTTTGAC-3′	
Reverse	5′-GTCCACCACCCTGTTGCTGTA-3′	

Table 1B
List of the four sense and antisense sequences of siRNA targeting Cav1 or p38α^{MAPK}

siGENOME SMART pool M-003467-01, Human Cav1, NM_001753	
Sense sequence 1	CUAAACACCUCAACGAUGAUU
Antisense sequence 1	5′-PUCAUCGUUGAGGUGUUUAGUU
Sense sequence 2	GCAGUUGUACCAUGCAUUAUU
Antisense sequence 2	5′-PUAAUGCAUGGUACAACUGCUU
Sense sequence 3	AUUAAGAGCUUCCUGAUUGUU
Antisense sequence 3	5′-PCAAUCAGGAAGCUCUUAAUUU
Sense sequence 4	GCAAAUACGUAGACUCGGAUU
Antisense sequence 4	5′-PUCCGAGUCUACGUAUUUGCUU
siGENOME SMART pool M-003512-05, Human MAPK14 (p38α^{MAPK}), NM_001315	
Sense sequence 1	CAAGGUCUCUGGAGGAAUUUU
Antisense sequence 1	5′-PAAUUCCUCCAGAGACCUUGUU
Sense sequence 2	GUCAGAAGCUUACAGAUGAUU
Antisense sequence 2	5′-PUCAUCUGUAAGCUUCUGACUU
Sense sequence 3	CGGCUUAUCUCAUUAACAGUU
Antisense sequence 3	5′-PCUGUUAAUGAGAUAAGCGGUU
Sense sequence 4	GUCCAUCAUUCAUGCGAAAUU
Antisense sequence 4	5′-PUUUCGCAUGAAUGAUGGACUU

0.1 mM, EGTA 0.1 mM (all from Merck), nonidet P-40 0.5% (Sigma–Aldrich, USA), dithiothreitol 1 mM (Amersham GE Healthcare, Sweden), and protease inhibitor), passed through a loose fitting Dounce homogenizer, and centrifuged at 3000 rpm for 3 min. Pellet (nuclear fraction) was washed with hypotonic lysis buffer before resuspension in the same buffer and sonication.

Western blots were performed as described in [22] using these antibodies: anti-caveolin1 mouse immunoglobulin G (IgG), anti-phospho-caveolin1 mouse IgG (BD Transduction Laboratories, Belgium), anti-caveolin1 rabbit IgG, anti-phospho-Akt (Ser 473) rabbit IgG, anti-Akt rabbit IgG, anti-phospho-Erk1/2 (Thr 202/Tyr 204) rabbit IgG, anti-Erk1 mouse IgG, anti-Erk2 mouse IgG, anti-phospho-L-CaD (Ser 789) rabbit IgG (Cell Signaling, USA), anti-Histone H1 mouse IgG, anti-p21 mouse IgG (Santa Cruz Biotechnology, USA), anti-α-tubulin mouse IgG (Clone B-5-1-2, Sigma–Aldrich) and horseradish peroxidase-linked secondary antibodies (anti-mouse antibodies: Amersham GE Healthcare). α-Tubulin and Histone H1 were used as reference protein for respectively, total and nuclear cell lysates analysis. Triplicates were performed.

2.4. Confocal microscopy

Cells were seeded at 20000 cells/cm^2 in MEM + 10% FBS on a glass cover slide. One day later, cells were fixed with paraformaldehyde 3% (Sigma–Aldrich). Cells were permeabilized with 1% Triton X-100. Specific mouse antibody Cav1 was added followed by the specific Alexa Fluor 488 anti-mouse conjugate antibody (Molecular Probes, USA). To visualize the nucleus, cells were incubated with TO-PRO-3 (ICN, USA). The glass cover slides were mounted in Mowiol (Sigma) and observed with a TCS confocal microscope (Leica, Germany) at constant multiplier. Triplicates were performed.

2.5. siRNA transfection

The si*GENOME* SMARTpool siRNA reagent M-003467-01 or M-003512-05 from Dharmacon (Belgium) were used to down-regulate the expression of Cav1 and p38α^{MAPK}, respectively. The sequences of siRNA are provided (Table 1B) and are guaranteed by the manufacturer. Non-targeting oligoribonucleotides (OR-0030-neg, Eurogentec, Belgium) were used as negative control. IMR-90 HDFs were seeded at half-confluency (20×10^3 cells/cm^2). One day later, the cells were transfected for 36 h, starting at day −3 before treatment with H_2O_2, with siRNA at 50 nM in MEM medium using Dharmafect transfection reagent. Cells were plated at day −1 before treatment with H_2O_2 (Fig. 2A). Cell viability was evaluated using the classical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay (MTT) (Sigma–Aldrich, Germany) [25].

3. Results

3.1. Increased abundance of total and phosphorylated Cav1 after treatment of IMR-90 HDFs with H_2O_2 at sublethal concentration

IMR-90 HDFs were treated for 2 h with H_2O_2 at 150 μM. This represents a sublethal treatment as shown earlier [5,6,21]. After the treatment with H_2O_2 at day 0, the cells exhibited a decrease of proliferative potential from day 2 to 3 and an increase in proportion of cells stained positive for SA β-gal at 3 days after the treatment, indicating that the cells underwent senescence [1,6,21,22] (data not shown).

No difference for Cav1 mRNA abundance could be detected by RT-real time PCR from 4 h to 3 days after the 2 h-treatment with H_2O_2, except a slight decrease at one day after treatment (Fig. 1A). As for Cav1 total protein abundance, Western blots showed an increase from 1 day to 3 days after treatment with H_2O_2, compared to non-treated controls (Fig. 1B). From day 1 to day 3 after the treatment with H_2O_2, the phospho-Cav1 abundance increased in parallel to the total abundance of Cav1. Independent of these variations, H_2O_2 induced the phosphorylation of Cav1 after 1 and 2 h of treatment and for 8 h after treatment with H_2O_2, compared with non-treated controls.

3.2. Nuclear localization of Cav1 in IMR-90 HDFs

Cav1 is often described as a plasma membrane protein [26]. We analyzed the subcellular localization of Cav1 at day 1 and 2 after H_2O_2 treatment. Surprisingly, the analysis of immunolabeled Cav1 showed a cytoplasmic and a nuclear localization in IMR-90 HDFs (Fig. 1C). An increase in the protein abundance was also observed, confirming the Western blots analysis presented at Fig. 1B.

To confirm the localization of Cav1, we analyzed cytoplasmic and nuclear extracts by Western blot. Cav1 was found in both cytoplasm and in nucleus (Fig. 1D). H_2O_2 treatment increased the abundance of Cav1 in the nuclear and cytoplasmic extracts. α-Tubulin was used as cytoplasmic protein marker and was absent in the purified nuclear extracts. Histone H1 was detected as nuclear marker and was not detected in the cytoplasmic extracts. These results were obtained with two different anti-Cav1 specific antibodies directed towards different antigenic areas of Cav1 sequence. To our knowledge, this is the first time that nuclear localization of Cav1 is reported in human fibroblasts. The siRNA analysis presented beneath further confirmed these results.

3.3. Down-regulation of Cav1 expression

Since Cav1 is overexpressed after treatment with H_2O_2 and thus potentially regulates premature senescence, we used spe-

Download English Version:

https://daneshyari.com/en/article/2049005

Download Persian Version:

https://daneshyari.com/article/2049005

Daneshyari.com