FEBS Letters 584 (2010) 4672–4678

journal homepage: www.FEBSLetters.org

Inhibition of the JNK/Bim pathway by Hsp70 prevents Bax activation in UV-induced apoptosis

Hui Li, Lei Liu, Da Xing *, Wei R. Chen

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China

ARTICLE INFO

Article history:
Received 7 May 2010
Revised 13 September 2010
Accepted 11 October 2010
Available online 29 October 2010

Edited by Vladimir Skulachev

Keywords:
Heat shock protein 70
Bax
c-Jun N-terminal kinase
BimL
Ultraviolet irradiation

ABSTRACT

Here we studied the mechanism by which heat shock protein 70 (Hsp70) prevents Bax activation during ultraviolet (UV)-induced apoptosis. UV treatment led to c-Jun N-terminal kinase (JNK) phosphorylation, Bim redistribution and subsequent Bax activation. Bim depletion caused a smaller reduction in apoptosis than that by JNK inhibition, indicating that Bim activation is not entirely responsible for induction of apoptosis and other mechanisms are involved. Hsp70 knockdown resulted in high levels of activated JNK and Bax, while Hsp70 overexpression inhibited these processes. These findings demonstrate that Hsp70 prevented Bax activation via inhibiting the JNK/Bim pathway. Simultaneously, increased binding of Hsp70 to Bax was observed. Collectively, our results for the first time demonstrate that Hsp70 prevents Bax activation both by inhibiting the JNK/Bim pathway and by interacting with Bax in UV-induced apoptosis.

© 2010 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

1. Introduction

Apoptosis can be initiated by diverse forms of cell stress such as heat shock and ultraviolet (UV) irradiation [1]. The Bcl-2 family members play a critical role in regulating apoptosis [2]. Bcl-2 family comprises three subfamilies: (a) antiapoptotic members, such as Bcl-2/Bcl-XL; (b) proapoptotic members, such as Bax, Bak, and Bok; and (c) BH3-only proteins, such as Bid, Bim, Puma, and Bmf [3]. The proapoptotic protein Bax plays an important role in apoptosis [4]. Additionally, the c-Jun N-terminal kinase (JNK) signaling pathway promotes Bax activation by phosphorylating Bim, suggesting that Bim provides a molecular link between the JNK signaling pathway and the Bax-dependent mitochondrial apoptotic machinery [5]. Following exposure to an apoptotic stimulus, Bax undergoes a conformational change, leading to exposure of its N- and C-termini and to its mitochondrial targeting. Within the mitochondrial membrane, oligomerized Bax facilitates mitochondrial membrane permeabilization, leading to cytochrome *c* release from mitochondria [4,6]. However, cells have self-repairing system to suppress apoptosis under harmful conditions, which can be accomplished by members of the heat shock protein family [7].

Heat shock proteins (Hsps) are a set of highly conserved proteins and they function as molecular chaperones. A well-characterized subgroup of Hsps is the heat shock protein 70 (Hsp70) family [8]. There are several Hsp70 family members, including stress-inducible Hsp70, constitutively expressed Hsp70 (Hsc70), mitochondrial Hsp75, and GRP78 [9]. The expression of Hsp70 can be induced by a variety of stresses, including heat shock, UV irradiation and oxidative stress [8]. Hsp70 has been reported to protect cells from apoptosis induced by various stresses and agents [10]. It can block the apoptotic pathway at different levels [11]. Most importantly, recent studies have suggested that Hsp70 prevents Bax translocation to mitochondria and blocks mitochondrial membrane permeabilization [12–15], although its molecular mechanisms are not clear at present.

The aim of this study is to investigate how Hsp70 inhibits Bax activation in UV-induced apoptosis. To determine the molecular mechanisms involved in this process, this study focuses on: (i) the activation of the JNK/Bim/Bax signaling pathway after UV irradiation; (ii) inhibitory effects of Hsp70 on the JNK/Bim/Bax pathway in UV-induced apoptosis; (iii) the interaction between Hsp70 and Bax.

2. Materials and methods

2.1. Materials and plasmids

We used antibodies against Hsp70, JNK and Bax (Cell Signaling Technology) and p-JNK (BD Biosciences). CFP-Bax was provided by

Abbreviations: LCSM, laser confocal scanning microscopy; JNK, c-Jun N-terminal kinase; CCK-8, Cell Counting Kit-8; CFP, YFP, DsRed and GFP, cyan, yellow, red and green fluorescent protein; FRET, fluorescence resonance energy transfer; Hsp70, heat shock protein 70; UV, ultraviolet; shRNA, short hairpin RNA

* Corresponding author. Fax: +86 20 85216052.
E-mail address: xingda@scnu.edu.cn (D. Xing).

0014-5793/$36.00 © 2010 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2010.10.050

Drs. Streuli and Gilmore (University of Manchester), YFP-Hsp70 was a gift from Dr. Morimoto of Northwestern University, and pDsRed-Mit was supplied by Dr. Gotoh (University of Yokyo). Hsp70 short hairpin RNA (shRNA) and Scr were provided by Dr. Tolkovsky [16]. The oligonucleotides for shRNA Bim were purchased from GenePharma (Shanghai, China) and were used as previously described [17]. GFP-BimL was generated as previously described [18]. Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Cell culture and treatments

The human lung adenocarcinoma cell line (ASTC-a-1) was cultured in DMEM supplemented with 15% fetal calf serum (FCS), penicillin (100 units/ml), and streptomycin (100 mg/ml) at 37 °C with 5% CO_2 in a humidified incubator. Transfection was performed with Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Cells were examined at 24–48 h after transfection. Before the 120 mJ/cm^2 UV treatment, medium was removed and collected, and then cells were rinsed with phosphate buffered saline. The medium was restored after treatment. For experiments with the inhibitor, cells were pretreated with 20 μM SP600125 (a specific inhibitor of JNK, Sigma, St. Louis, MO, USA) for 1 h before UV irradiation. SP600125 was kept in the medium throughout the experimental process.

2.3. Cell viability assays

ASTC-a-1 cells were cultured in a 96-well microplate at a density of 5×10^3 cells/well for 24 h. Cell viability was assessed with Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) at indicated times post UV treatment. OD450, the absorbance value at 450 nm, was read with a 96-well plate reader (DG5032, Huadong, Nanjing, China), to determine the viability and proliferation of the cells.

2.4. Flow cytometry

Annexin V-fluorescein isothiocyanate (FITC; 0.1 μg/ml) was used for the assessment of phosphatidylserine exposure. Propidium iodide (PI; 0.5 μg/ml) was used for cell viability analysis. Cell death was measured in a FACSCanto™ II cytofluorimeter (Becton Dickinson, Mountain View, CA). Compensation was used wherever necessary.

2.5. Subcellular fractionation

Cytosolic and mitochondria-enriched fractions were prepared using Subcellular Proteome Extraction Kit (ProteoExtract™, Calbiochem, Darmstadt, Germany) according to the manufacturer's instructions.

2.6. Bax conformational change analysis

Cells were lysed with ice-cold lysis buffer (150 mM NaCl, 10 mM HEPES (pH 7.4), 1% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonic acid, and 100 μg/ml PMSF) containing protease inhibitors. For immunoprecipitation, 2.5 μg of anti-Bax 6A7 monoclonal antibody (Abcam, Cambridge) was added into 500 μg of cell lysate. The obtained immune complexes were subjected to western blotting analysis with anti-Bax polyclonal antibody.

2.7. Laser confocal scanning microscopy (LCSM) and fluorescence resonance energy transfer (FRET) acceptor photo-bleaching technique

Fluorescence of cyan fluorescent protein (CFP), green fluorescent protein (GFP), yellow fluorescent protein (YFP), red fluorescent protein (DsRed), and Mitotracker were monitored confocally with LCSM, using different excitation wavelengths and detection filters as previously described [19].

FRET acceptor photo-bleaching was performed on LCSM to detect the interaction between YFP-Hsp70 and CFP-Bax. For excitation, the 458 nm line of an argon-ion laser was attenuated with an acousto-optical tunable filter and reflected by a dichroic mirror (main beam splitter HFT458), and focused through a Plan-Neofluar 40 ×/1.3 NA oil DIC objective (Carl Zeiss) onto the sample. CFP (the donor) and YFP (the FRET acceptor) emissions were collected through 470–500 and 535–545 nm band pass filters, respectively. YFP was excited at 514 nm, and its emission was detected with 565 to 615 nm band-pass (YFP channel). We bleached the YFP signal (the acceptor) in a certain area within the cell (identified by a rectangle) with 514 nm line of an argon-ion laser at 100% power for 300 iterations.

2.8. CFP-Bax and GFP-BimL translocation assay

To monitor Bax translocation in living cells, cells were transfected with CFP-Bax and were stained by MitoTracker for mitochondrial labeling. The cells exhibiting strong punctuate staining of CFP, which overlapped with the distribution of MitoTracker, were counted as the cells with mitochondrially localized Bax. The analysis of GFP-BimL mitochondrial translocation was similar to that of Bax.

2.9. Co-immunoprecipitation and western blotting assays

Cells were lysed with ice-cold lysis buffer (50 mM Tris–HCl [pH 8.0], 150 mM NaCl, 1 × TritonX-100, 100 μg/ml PMSF and Protease Inhibitor Cocktail Set I) for 45 min on ice. After centrifugation, the supernatant was incubated with the antibody against Bax and subsequently with protein A-Sepharose (50% slurry) at 4 °C overnight. After washed five times, pellet was resuspended with the same volume of SDS sample buffer, and boiled to remove Sepharose beads. Then the cell lysates and immunoprecipitates were analyzed by western blotting [20].

3. Results

3.1. Hsp70 confers resistance against UV-induced apoptosis

To study Hsp70 expression after UV irradiation, western blotting analysis was performed. The results show that the expression of Hsp70 increased gradually (Fig. 1A). To investigate the cytoprotective function of Hsp70 after UV irradiation, cell viability was analyzed using CCK-8. Overexpressed Hsp70 clearly reduced the level of cell death, compared with the UV-only treatment (Fig. 1B). In addition, western blotting was performed to confirm Hsp70 overexpression (Fig. 1B).

We further studied cell apoptosis using flow cytometry after knocking down Hsp70 utilizing RNA interference approach. Scr was used as control. The data show that silencing Hsp70 increased cell apoptosis (Fig. 1C). Statistical results of apoptotic cells under different treatments are given in Fig. S1 (Supplementary information) blotting was also performed to confirm Hsp70 knockdown (Fig. S1). These results clearly suggest that Hsp70 has distinct cytoprotective function in UV-induced apoptosis.

3.2. Hsp70 prevents Bax mitochondrial translocation

Generally, the activation of Bax is inferred by its translocation from cytosol to mitochondria. UV-induced Bax mitochondrial translocation, as well as the activation of Bax, was investigated

Download English Version:

https://daneshyari.com/en/article/2049230

Download Persian Version:

https://daneshyari.com/article/2049230

Daneshyari.com