Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 358 (2007) 559–565
BBRC
www.elsevier.com/locate/ybbrc

BimL involvement in Bax activation during UV irradiation-induced apoptosis

Miaojuan Chen, Da Xing *, Tongsheng Chen, Lan Zhang

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

Received 22 April 2007
Available online 4 May 2007

Abstract

Bax, a proapoptotic member of the Bcl-2 family, localizes largely in the cytoplasm but translocates to mitochondria and undergoes oligomerization to induce the release of apoptogenic factors in response to apoptotic stimuli. However, the molecular mechanism of Bax activation is not fully understood. We show here the role of BimL in Bax activation during UV irradiation-induced apoptosis. In this study, GFP-BimL plasmid was constructed. The dynamic interaction between BimL and Bax during UV irradiation-induced apoptosis was observed using fluorescence resonance energy transfer (FRET) technique. Our experimental results showed that BimL translocation to mitochondria occurred before Bax translocation, and that BimL activated Bax indirectly. Moreover, inhibition of c-Jun N-terminal protein kinase (JNK) activation blocked BimL translocation, delayed and attenuated Bax translocation and subsequent apoptosis. These results demonstrate that BimL is involved in UV irradiation-induced apoptosis by indirectly activating Bax.
© 2007 Elsevier Inc. All rights reserved.

Keywords: UV irradiation; Apoptosis; JNK; BimL; Bax; FRET

Bax is a proapoptotic member of the Bcl-2 family of proteins that is implicated in the pathogenesis of cell death in an increasing number of models of apoptosis both *in vivo* and *in vitro*. Bax is constitutively present in many cell types that undergo apoptosis in response to a variety of stimuli. By contrast, in other cells Bax expression is induced by activation of p53 upon damage to the genome or interference with the normal progression of the cell cycle [1]. Whether constitutively expressed or induced, however, the primary action of Bax is a consequence of its translocation from the cytosol to the mitochondria. Translocation of preformed Bax from the cytosol to the mitochondria has been reported with a variety of apoptotic stimuli [2–6]. In the situation where Bax is synthesized upon introduction of an apoptotic stimulus, evidence exists that a similar mechanism of Bax activation is operative to control its translocation to the mitochondria [7]. Current knowledge about apoptotic signaling cascades supports the view that BH3-only molecules initiate the pathway by activating the downstream effector Bax to trigger mitochondrial apoptosis [8].

The findings of the release of Bim from microtubule-associated dynein motor complexes and relocation to mitochondria after UV irradiation suggest that Bim acts as an important factor in induction of Bax activation and apoptosis [9]. Currently, three major Bim isoforms have been characterized: BimS, BimL, and BimEL [10]. The short isoform (BimS) potently induces apoptosis and is normally only transiently expressed in cells during apoptosis. BimEL and BimL are expressed in a variety of tissues and cell types, normally associated with the microtubule–dynein complex, and released after death stimulation. However, how Bim activates Bax to induce apoptosis, directly or indirectly, is controversial [11,12].

Fluorescence resonance energy transfer (FRET) technique has been widely used to study protein–protein

Abbreviations: FRET, fluorescence resonance energy transfer; MCF7, human breast cancer cells; JNK, c-Jun N-terminal protein kinase; CCK-8, Cell Counting Kit-8; mPTP, mitochondria permeability transition pore; VDAC, voltage-dependent anion channel; PDT, photodynamic therapy.

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

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.04.167

interactions in living cells with improved spatial and temporal resolution, distance range, and sensitivity [13–16]. In this study, confocal microscopy and FRET were used to monitor the dynamic interaction between BimL and Bax after UV irradiation treatment at the single living cell level, and to determine the role of BimL in Bax activation during UV irradiation-induced apoptosis. Our findings extend the knowledge about the cellular signaling mechanisms mediating UV irradiation-induced apoptosis.

Materials and methods

Plasmid construction. BimL cDNA was obtained from rat pheochromocytoma cells total RNA by RT-PCR technique. The BimL cDNA was amplified with Pfu polymerase using the special primers introducing NheI site, and BamHI site and confirmed by sequencing. The down primer was designed to remove the stop codon of the BimL gene. The special primers: UP 5′-ACACGCTAGCTATGGCCAAGCAACCTTCTGA-3′, DW 5′-ACACGGATCCACATGCCTTCTCCATACCAGAC-3′. The product was cloned into NheI and BamHI sites of the pEGFP-N1 plasmid and subsequently sequenced. DsRed-Mit was presented by Prof. Yukiko Gotoh [17]. DsRed-Mit, a molecular fluorescence probe, can localize at mitochondria selectively. YFP-Bax was presented by Prof. Charles and Andrew [18].

Cell culture and treatments. Human breast cancer cells (MCF7) were cultured in DMEM (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml) with 5% CO_2 at 37 °C in humidified incubator. Transient transfection of MCF7 cells was performed with Lipofectamine reagent according to the manufacturer's protocol. Apoptosis was induced by UV irradiation (253.7 nm, 200 μW/cm^2) at fluence of 100 mJ/cm^2. For UV irradiation, medium was removed, and cells were rinsed with PBS and irradiated, and then medium was restored. Cells were pretreated with 20 μM JNK inhibitor SP600125 (Sigma, St. Louis, MO, USA) for 1 h before UV irradiation. SP600125 was kept in the medium throughout the experimental process.

Confocal microscopy. To observe the activation of Bax, MCF7 cells cotransfected with YFP-Bax and DsRed-Mit were treated with UV irradiation. A Laser Scanning Microscopes combination system (LSM510/ConfoCor2, Zeiss, Jena, Germany) with 100× oil immersion planapochromat objective lens was used. Cells were maintained at 37 °C using the temperature regulator (Tempcontrol 37-2 digital, Zeiss). Temperature elevations of culture liquid were less than 0.2 °C throughout the experimental process. Confocal images of YFP-Bax fluorescence from the cells were obtained using a 514 nm excitation light from an argon laser and a 565–615 nm band-pass filter. Images of DsRed-Mit fluorescence from the cells were obtained using a 543 nm excitation light from a He–Ne laser and a 600–650 nm band-pass filter. To observe the activation of BimL, MCF7 cells cotransfected with GFP-BimL and DsRed-Mit were treated with UV irradiation. Confocal images of GFP-BimL fluorescence from the cells were obtained using a 488 nm excitation light from an argon laser and a 500–550 nm band-pass filter.

Cells showing Bax translocation were scored under an inverted fluorescence microscope using the 20× objective.

FRET Image acquisition and data analysis. To investigate the dynamic interaction between BimL and Bax, MCF7 cells cotransfected with GFP-BimL and YFP-Bax were treated with UV irradiation with or without SP600125 (20 μM) pretreatment, and observed using laser scanning microscopes. SP600125 has been shown to be a highly selective inhibitor of JNK-1, -2, -3 [19]. Recently reports showed that Bim activation is dependent on JNK phosphorylation [20,21]. Therefore, inhibition of JNK activation may block BimL activation. To measure the FRET effect, cells were excited by the 458 nm line of an Ar–Ion Laser. The emission fluorescence was split by a second dichroic mirror (secondary beam splitter NFT545) into two separate channels: the 480–520 nm band-pass (GFP channel) and the 565–615 nm band-pass (FRET channel). In addition, YFP was excited at 514 nm, and YFP emission was detected with 565–615 nm band-pass (YFP channel). To quantify the results, the images of GFP and YFP emission intensities were processed with Zeiss Rel3.2 image processing software (Zeiss, Germany).

Cell viability assays. MCF7 cells were cultured in a 96-well microplate at a density of 4×10^3 cells/well for 24 h. The cells were divided into two groups. One group was pretreated with SP600125 for 1 h before UV irradiation, while the other not. Then both groups were treated with UV irradiation. After UV irradiation, 96-well microplates were returned to the incubator for a further culture. Cell cytotoxicity assay was assessed with Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) at 0, 6, 12 and 24 h after UV irradiation, respectively. OD_{450}, the absorbance value at 450 nm, was read with a 96-well plate reader (DG5032, Hua dong, Nanjing, China), and the OD_{450} is proportional inversely to the degree of cells apoptosis.

Results

Bax translocation to mitochondria during UV irradiation-induced apoptosis

To investigate the activation of BimL during UV irradiation-induced apoptosis, MCF7 cells cotransfected with YFP-Bax and DsRed-Mit were treated with UV irradiation to induce apoptosis. Using confocal microscope, we monitored the activation of Bax in normal cells or during UV irradiation-induced apoptosis. In normal cells or before UV irradiation, Bax distributed evenly in both cytoplasm and nucleus (Supplementary Fig. 1). However, after UV irradiation Bax translocated to mitochondria and eventually formed clusters associated with mitochondria (Fig. 1A). As shown in Fig. 1B, the percentage of cells showing Bax translocation was 29% ± 5.67%, 49.5% ± 3.54%, 90% ± 1.41% at 6, 12, and 18 h after UV irradiation, respectively. The results showed that Bax translocation increased progressively during the period of 18 h after UV irradiation. Together, the results from Fig. 1 indicated that UV irradiation induced Bax translocation to mitochondria.

BimL translocation to mitochondria during UV irradiation-induced apoptosis

To investigate the activation of BimL during UV irradiation-induced apoptosis, MCF7 cells cotransfected GFP-BimL and DsRed-Mit were treated with UV irradiation to induce apoptosis. Confocal microscopy revealed that BimL had a cytoplasmic distribution before UV irradiation or in normal cells (Supplementary Fig. 2), while BimL translocated to mitochondria and colocalized with mitochondria after UV irradiation (Fig. 2A). In order to demonstrate BimL translocation more clearly, we magnified the images within Fig. 2A, and showed them in Fig. 2B. From these magnified images, it is clearly evident that BimL did not reside on mitochondrial before UV irradiation or in normal cells. Instead, BimL translocated to mitochondria after UV irradiation. These results implied that BimL was involved in UV irradiation-induced apoptosis.

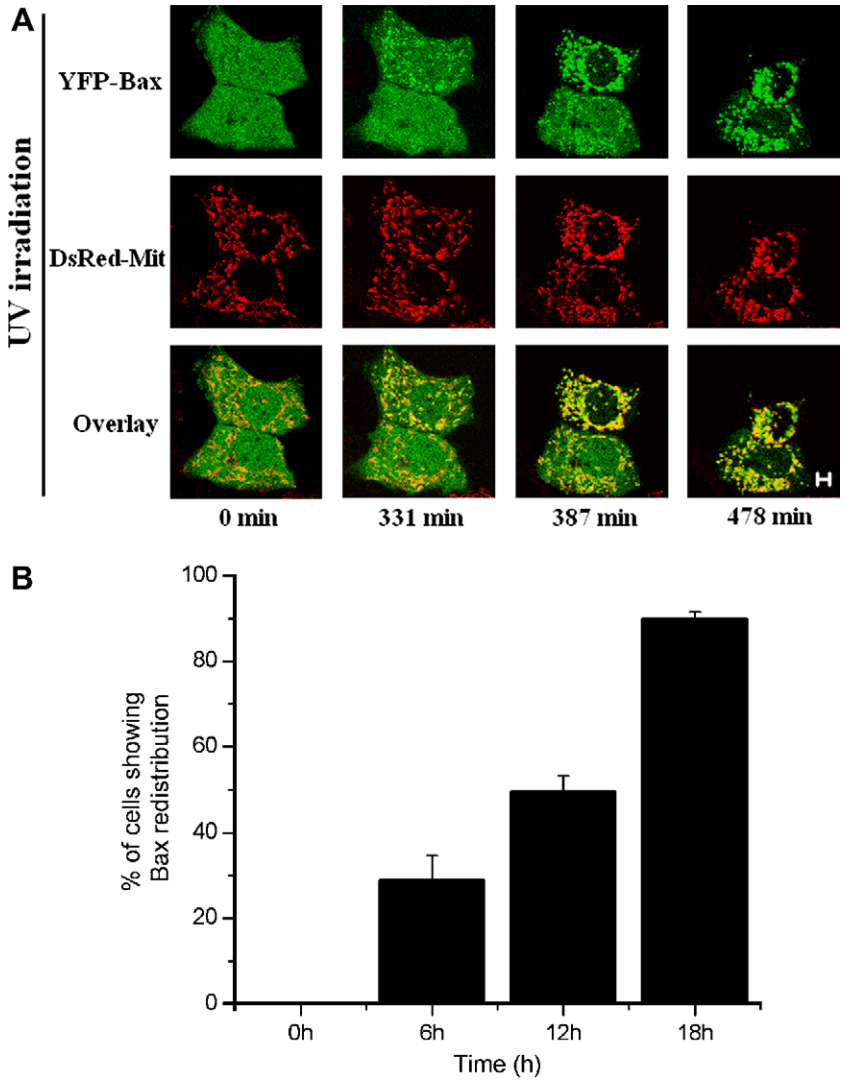

Fig. 1. Bax translocation to mitochondria during UV irradiation-induced apoptosis. MCF7 cells were cotransfected with YFP-Bax and DsRed-Mit before UV irradiation. (A) After UV irradiation (100 mJ/cm^2), Bax translocated to mitochondria, and eventually formed clusters. Bar, 5 μm. (B) Quantitation of cells showing Bax translocation after UV irradiation. At the indicated time points the proportion of cells showing Bax translocation was determined *in vivo* by counting under an inverted fluorescence microscope. At least 100 cells were scored for each experimental point. Columns represent the percentage of cells showing Bax translocation. Error bars show SDs of percentages obtain by counting at least four different fields. Similar results were obtained from three separate experiments.

Dynamic interaction between BimL and Bax during UV irradiation-induced apoptosis

FRET was used to monitor the dynamic interaction between BimL and Bax in MCF7 cells cotransfected with GFP-BimL and YFP-Bax after UV irradiation. As shown in Fig. 3A, before UV irradiation, GFP-BimL had a cytoplasmic distribution, and YFP-Bax distributed evenly in both cytoplasm and nucleus. However, after UV irradiation, BimL translocated to mitochondria, which occurred before Bax translocation. Eventually BimL and Bax both formed clusters. To quantitatively determine the kinetics of GFP-BimL and YFP-Bax activation, the time-dependent fluorescence intensities of GFP-BimL and YFP-Bax in a cellular subregion were shown in Fig. 3B. It appeared that BimL translocation occurred before Bax translocation. The time-course YFP/GFP ratio images were shown in Fig. 3A. The images and the data showed that FRET between Bax and BimL remained unchanged throughout the observation period (Fig. 3C). The results indicated that BimL did not activate Bax directly during UV irradiation-induced apoptosis.

Inhibition of JNK activation blocked BimL translocation, delayed and attenuated Bax translocation and subsequent apoptosis

In order to determine the role of BimL in UV irradiation-induced Bax activation, MCF7 cells expressing YFP-Bax and GFP-BimL were pretreated with SP600125 (20 μM) before UV irradiation. In the presence of SP600125, BimL remained distributing in cytoplasm

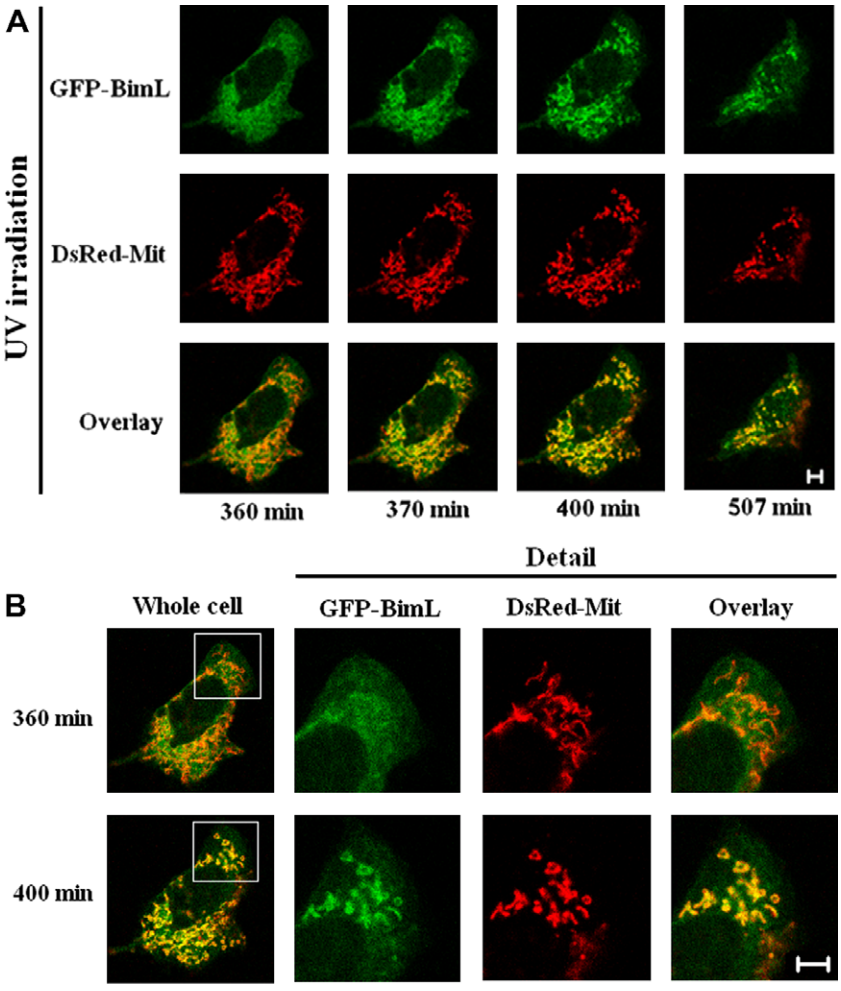

Fig. 2. BimL translocation to mitochondria during UV irradiation-induced apoptosis. MCF7 cells were cotransfected with GFP-BimL and DsRed-Mit before UV radiation. (A) After UV irradiation (100 mJ/cm^2), BimL translocated to mitochondria and colocalized with mitochondria. Bar, 5 μm. (B) The detail of the localization of BimL during UV irradiation-induced apoptosis. Bar, 5 μm. Similar results were obtained from three separate experiments.

throughout the observation period, while Bax translocated to mitochondria about 400 min after UV irradiation (Fig. 4A). To quantitatively determine the kinetics of GFP-BimL and YFP-Bax activation, we plotted the time-dependent fluorescence intensity changes of GFP-BimL and YFP-Bax in a cellular subregion, respectively (Fig. 4B). It appeared that the activation of BimL was blocked by inhibition of JNK activation. Moreover, cells showing Bax translocation were quantified after UV irradiation with or without SP600125 pretreatment (Supplementary Fig. 3A). In the presence of SP600125, the percentage of cells showing Bax translocation significantly decreased to $19 \pm 1.41\%$, $32.5 \pm 3.54\%$, $68 \pm 2.19\%$ at 6, 12, and 18 h after UV irradiation. The results showed that Bax translocation was delayed and attenuated by inhibition of JNK activation. This implied that BimL is an upstream factor in induction of Bax activation.

To further confirm the influence of inhibition of BimL translocation on UV irradiation-induced apoptosis, we used CCK-8 to observe the cell viability after UV irradiation with or without SP600125 pretreatment. The results showed that the apoptosis induced by UV irradiation was partly blocked by inhibition of JNK activation (Supplementary Fig. 3B).

Taken together, these results indicated that inhibition of JNK activation blocked BimL translocation, delayed and attenuated Bax translocation and subsequent apoptosis, which implied that BimL was one of upstream factors of Bax activation during UV irradiation-induced apoptosis.

Discussion

BH3-only proteins have been shown to be upstream regulators of Bax/Bak activation [22]. However, the mechanisms through which BH3-only proteins regulate Bax/Bak activation remain uncertain. In this study, we show the role of BimL in Bax activation during UV irradiation-induced apoptosis. We monitored for the first time the dynamics of BimL activation during UV irradiation-induced apoptosis in living cells. Our results showed that BimL translocated to mitochondria during UV irradia-

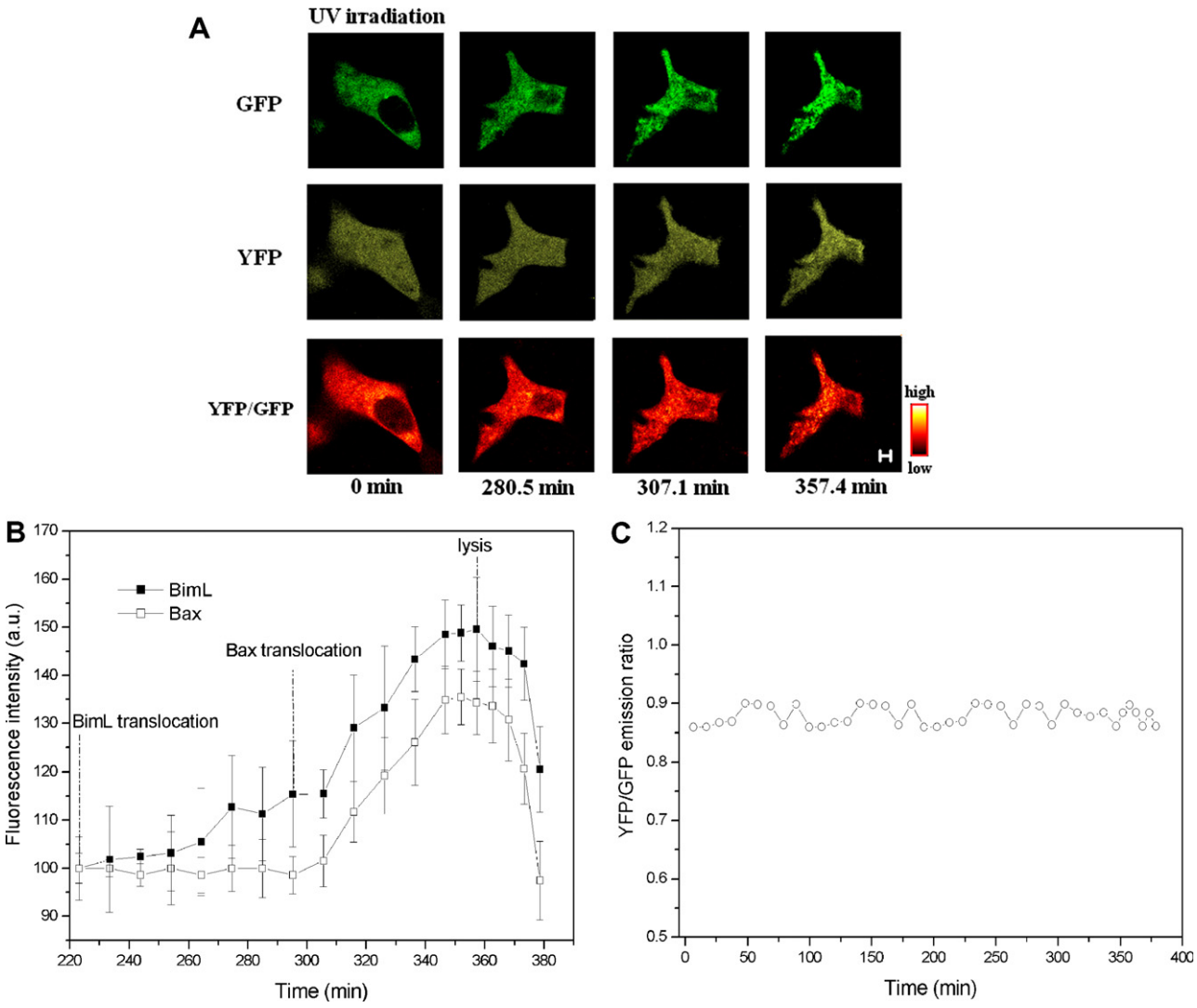

Fig. 3. Dynamic interaction between BimL and Bax during UV irradiation-induced apoptosis. MCF7 cells cotransfected with YFP-Bax and GFP-BimL were treated with UV irradiation (100 mJ/cm^2) and imaged by confocal microscopy. (A) Bax and BimL distribution were imaged simultaneously in a single cell. The images of YFP/GFP ratio were recorded with LSM microscope and processed with pseudocolor technique. Bar, 5 μm. (B) The intensities of Bax emission and BimL emission in a cellular subregion correspond to the data in (A). (C) Dynamics of YFP/GFP ratio correspond to the data in (A). Similar results were obtained from three separate experiments.

tion-induced apoptosis (Fig. 2). Moreover, our observations showed that inhibition of BimL activation (Fig. 4) delayed and attenuated Bax translocation and subsequent apoptosis (Supplementary Fig. 3). These results suggest BimL is involved in Bax activation during UV irradiation-induced apoptosis. However, Bax translocation and subsequent apoptosis were not completely blocked by inhibition of BimL translocation (Supplementary Fig. 3). These results indicated that BimL is not the only upstream factor of UV irradiation-induced Bax activation. Previous studies showed that UV irradiation was a DNA-damage agent that activates a p53-dependent apoptotic response [23,24]. p53 has been shown to directly activate Bax to mediate mitochondrial membrane permeabilization and apoptosis [25]. Therefore, UV irradiation-induced Bax activation has multiple upstream regulators to trigger together.

Our study found that BimL did not directly activate Bax during UV irradiation-induced apoptosis (Fig. 3). Thus, what is the possible mechanism through which BimL activates Bax? One possibility is that Bim promotes mitochondrial apoptosis by interacting with and neutralizing anti-apoptotic members of the Bcl-2 family (e.g., Bcl-2 and Bcl-xL) [26]. Antagonism of Bcl-2/Bcl-xL triggers an oxidative stress-dependent opening of a mitochondrial permeability channel that activates Bax translocation and oligomerization to induce apoptosis [27,28]. Our results showed that Bcl-xL bound Bax directly and inhibited Bax translocation and oligomerization in UV irradiation-induced apoptosis (data not shown). The results implied that Bim might interact with Bcl-2/Bcl-xL directly to release Bax from the sequestration. The latter effect could indirectly promote Bax oligomerization, insertion into the

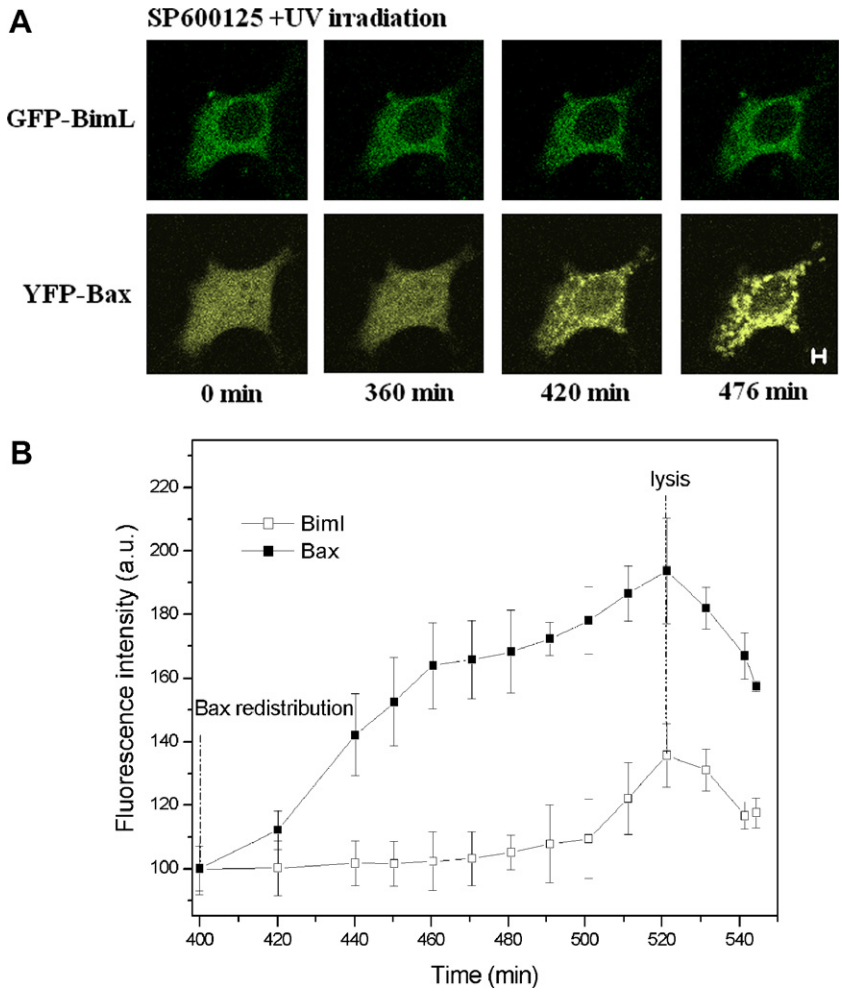

Fig. 4. Inhibition of JNK activation blocked BimL translocation. MCF7 cells expressing YFP-Bax and GFP-BimL were pretreated with SP600125 (20 μM) before UV irradiation (100 mJ/cm^2). (A) Bax and BimL distribution were imaged simultaneously in a single cell. Bar, 5 μm. (B) The intensities of Bax emission and BimL emission in a cellular subregion correspond to the data in (A). Similar results were obtained from three separate experiments.

mitochondrial membrane, and an ensuing mitochondrial permeabilization. Furthermore, recent reports have shown that Bim interacts with components of the mitochondria permeability transition pore (mPTP). For example, Bim has recently been shown to interact with the voltage-dependent anion channel (VDAC) in isolated mitochondrial preparations, leading directly to opening of the mPTP and mitochondrial depolarization [29]. Lastly, one should consider the possibility that Bim may have multiple cellular targets, perhaps including some combination of Bcl-2/Bcl-xL, VDAC or ANT, Bax or Bak, and other, as yet, unidentified proteins, so these interdependent interactions may ultimately trigger cell death.

Bim activation is dependent on JNK phosphorylation [20,21]. Our results have indicated that inhibition of JNK activation blocked BimL translocation (Fig. 4). Therefore, Bim as the target of the JNK signaling pathway provides a molecular link between JNK and the engagement of the mitochondrial cell death pathway in cells. This implied that Bim may be as an important target of JNK in response to other stimuli, such as photodynamic therapy (PDT). Although PDT inflicts damage to cells largely via reactive oxygen species, recent reports showed that PDT could initiate apoptosis via the activation of JNK signal pathway [30,31]. However, the mechanism by which JNK may cause the activation of BH3-only proteins is unclear. Bim may be as one potential target of JNK during PDT-induced apoptosis.

In summary, we examined the dynamic interaction between BimL and Bax during UV irradiation-induced apoptosis. To our best knowledge, this was the first time that the interaction between BimL and Bax has been observed by FRET during UV irradiation-induced apoptosis at the single cell level. Our results have demonstrated that BimL is involved in the UV irradiation-induced apoptosis by activating indirectly Bax.

Acknowledgments

We thank Prof. Yukiko Gotoh in the institute of molecular and cellular bioscience, university of Tokyo for providing the plasmid DsRed-Mit, and Prof. Charles and

Andrew in the school of biological science, university of Manchester for providing the plasmid YFP-Bax. This research is supported by the National Natural Science Foundation of China (30670507; 30600128; 30470494), and the Natural Science Foundation of Guangdong Province (015012).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.04.167.

References

[1] T. Choudhuri, S. Pal, M.L. Agwarwal, T. Das, G. Sa, Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction, FEBS Lett. 512 (2002) 334–340.
[2] Y.T. Hsu, K.G. Wolter, R.J. Youle, Cytosol-to-membrane redistribution of Bax and Bcl-X_L during apoptosis, Proc. Natl. Acad. Sci. USA 94 (1997) 3668–3672.
[3] S. Desagher, A. Osen-Sand, A. Nichols, R. Eskes, S. Montessuit, S. Lauper, K. Maundrell, B. Antonsson, J.C. Martinou, Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome *c* release during apoptosis, J. Cell Biol. 144 (1999) 891–901.
[4] R. Eskes, S. Desagher, B. Antonsson, J.C. Martinou, Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane, Mol. Cell. Biol. 20 (2000) 929–935.
[5] K.M. Murphy, V. Ranganathan, M.L. Farnsworth, M. Kavallaris, R.B. Lock, Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells, Cell Death Differ. 7 (2000) 102–111.
[6] A. Gross, J. Jockel, M.C. Wei, S.J. Korsmeyer, Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis, EMBO J. 17 (1998) 3878–3885.
[7] N.O. Karpinich, M. Tafani, R.J. Rothman, M.A. Russo, J.L. Farber, The course of etoposide-induced apoptosis from damage to DNA and p53 activation to mitochondrial release of cytochrome *c*, J. Biol. Chem. 277 (2002) 16547–16552.
[8] P. Bouillet, A. Strasser, BH3-only proteins-evolutionarily conserved proapoptotic Bcl-2 family members essential for initiating programmed cell death, J. Cell Sci. 115 (2002) 1567–1574.
[9] H. Puthalakath, D.C. Huang, L.A. O'Reilly, S.M. King, A. Strasser, The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex, Mol. Cell 3 (1999) 287–296.
[10] L. O'Connor, A. Strasser, L.A. O'Reilly, G. Hausmann, J.M. Adams, S. Cory, D.C. Huang, Bim: a novel member of the Bcl-2 family that promotes apoptosis, EMBO J. 17 (1998) 384–395.
[11] M. Marani, T. Tenev, D. Hancock, J. Downward, N.R. Lemoine, Identification of novel isoforms of the BH3 domain protein Bim which directly activate Bax to trigger apoptosis, Mol. Cell. Biol. 22 (2002) 3577–3589.
[12] Y. Zhu, B.J. Swanson, M. Wang, D.A. Hildeman, B.C. Schaefer, X. Liu, H. Suzuki, K. Mihara, J. Kappler, P. Marrack, Constitutive association of the proapoptotic protein Bim with Bcl-2-related proteins on mitochondria in T cells, Proc. Natl. Acad. Sci. USA 101 (2004) 7681–7686.
[13] S. Fields, O. Song, A novel genetic system to detect protein–protein interactions, Nature 340 (1989) 245–246.
[14] K. Takemoto, T. Nagai, A. Miyawaki, M. Miura, Spatio-temporal activation of caspase revealed by indicator that is insensitive to environmental effects, J. Cell. Biol. 160 (2003) 235–243.
[15] F. Wang, T.S. Chen, D. Xing, J.J. Wang, Y.X. Wu, Measuring dynamics of caspase-3 activity in living cells using FRET technique during apoptosis induced by high fluence low-power laser irradiation, Lasers Surg. Med. 36 (2005) 2–7.
[16] Y. Wu, D. Xing, W.R. Chen, Single cell FRET imaging for determination of pathway of tumor cell apoptosis induced by photofrin-PDT, Cell Cycle 5 (2006) 729–734.
[17] F. Tsuruta, N. Masuyama, Y. Gotoh, The Phosphatidylinositol 3-kinase (PI3K)-Akt pathway suppresses Bax translocation to mitochondria, J. Biol. Chem. 277 (2002) 14040–14047.
[18] A.J. Valentijn, A.D. Metcalfe, J. Kott, C.H. Streuli, A.P. Gilmore, Spatial and temporal changes in Bax subcellular localization during anoikis, J. Cell Biol. 162 (2003) 599–612.
[19] B.L. Bennett, D.T. Sasaki, B.W. Murray, E.C. O'Leary, S.T. Sakata, W. Xu, J.C. Leisten, A. Motiwala, S. Pierce, Y. Satoh, S.S. Bhagwat, A.M. Maning, D.W. Anderson, SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase, Proc. Natl. Acad. Sci. USA 98 (2001) 13681–13686.
[20] G.V. Putcha, S. Le, S. Frank, C.G. Besirli, K. Clark, B. Chu, S. Alix, R.J. Youle, A. Lamarche, A.C. Maroney, J.E. Johnson, JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis, Neuron 38 (2003) 899–914.
[21] K. Lei, R.J. Davis, JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis, Proc. Natl. Acad. Sci. USA 100 (2003) 2432–2437.
[22] A. Letai, M. Bassik, L. Walensky, M. Sorcinelli, S. Weiler, S. Korsmeyer, Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics, Cancer Cell 2 (2002) 183–192.
[23] A. Ziegler, A.S. Jonason, D.J. Leffell, J.A. Simon, H.W. Sharma, J. Kimmelman, L. Remington, T. Jacks, D.E. Brash, Sunburn and p53 in the onset of skin cancer, Nature 372 (1994) 773–776.
[24] K.H. Kraemer, Sunlight and skin cancer: another link revealed, Proc. Natl. Acad. Sci. USA 94 (1997) 11–14.
[25] J.E. Chipuk, T. Kuwana, L.B. Hayers, N.M. Droin, D.D. Newmeyer, M. Schuler, D.R. Green, Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis, Science 303 (2004) 1010–1014.
[26] A. Strasser, H. Puthalakath, P. Bouillet, D.C. Huang, L. O'Connor, L.A. O'Reilly, L. Cullen, S. Cory, J.M. Adams, The role of Bim, a pro-apoptotic BH3-only member of the Bcl-2 family in cell-death control, Ann. NY Acad. Sci. 917 (2000) 541–548.
[27] J.M. Scanlon, I.J. Reynolds, Effects of oxidants and glutamate receptor activation on mitochondrial membrane potential in rat forebrain neurons, J. Neurochem. 71 (1998) 2392–2400.
[28] S.B. Mukherjee, M. Das, G. Sudhandiran, C. Shaha, Increase in cytosolic Ca^{2+} levels through the activation of non-selective cation channels induced by oxidative stress causes mitochondrial depolarization leading to apoptosis-like death in Leishmania donovani promastigotes, J. Biol. Chem. 277 (2002) 24717–24727.
[29] T. Sugiyama, S. Shimizu, Y. Matsuoka, Y. Yoneda, Y. Tsujimoto, Activation of mitochondrial voltage-dependent anion channel by a pro-apoptotic BH3-only protein Bim, Oncogene 21 (2002) 4944–4956.
[30] L. Klotz, C. Fritsch, K. Briviba, N. Tsacmacidis, F. Schliess, H. Sies, Activation of JNK and p38 but not ERK MAP kinases in human skin cells by 5-aminolevulinate-photodynamic therapy, Cancer Res. 58 (1998) 4297–4300.
[31] Z. Tong, G. Singh, K. Valerie, A.J. Rainbow, Activation of the stress-activated JNK and p38 MAP kinases in human cells by photofrin-mediated photodynamic therapy, J. Photochem. Photobiol. B 71 (2003) 77–85.