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ORIGINAL PAPER

Bim_L displacing Bcl-x_L promotes Bax translocation during TNFα-induced apoptosis

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Abstract Bcl-2 family proteins are implicated as essential regulators in tumor necrosis factor-α (TNFα)-induced apoptosis. Bim_L, a BH3-only member of Bcl-2 family, can directly or indirectly activate the proapoptotic Bax and the subsequent mitochondrial apoptotic pathway. However, the molecular mechanism of Bim_L activating Bax activation during TNFα-induced apoptosis is not fully understood. In this study, the role of Bim_L in Bax activation during TNFα-induced apoptosis was investigated in differentiated PC12 and MCF7 cells, with real-time single-cell analysis. The experimental results show that Bax translocated to mitochondria and cytochrome c (Cyt c) released from mitochondria after TNFα treatment. Furthermore, SP600125 (specific inhibitor of JNK) could inhibit the Cyt c release from mitochondria. Co-immunoprecipitation results show that, the interaction between Bcl-x_L and Bax decreased after TNFα treatment, while that between Bcl-x_L and Bim_L increased. Bax did not co-immunoprecipitate with Bim_L before or after the TNFα treatment. In addition, the increased interaction between Bim_L and Bcl-x_L was dynamically monitored by using fluorescence resonance energy transfer (FRET) technique. Most importantly, there was no evidence of Bim_L redistribution to mitochondria until cell apoptosis. By comprehensively analyzing these data, it is concluded that Bim_L displaces Bcl-x_L in the mitochondria and promotes Bax translocation during TNFα-induced apoptosis.

Keywords TNFα · Apoptosis · Bax · Bcl-x_L · FRET · Bim_L

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Introduction

Bcl-2 family members are defined functionally as either proapoptotic or antiapoptotic based on their ability to impact cell survival and apoptosis. Each Bcl-2 family members characterized by the presence of one or more Bcl-2 homology (BH) domains [1, 2]. The family may be subdivided into three main groups based on regions of BH domains and function: multidomain antiapoptotic proteins (e.g., Bcl-2, Bcl-x_L, Bcl-w and Mcl-1), multidomain proapoptotic proteins (e.g., Bax and Bak), and BH3-only proapoptotic proteins (e.g., Bid, Bim, Bad, Bik, Noxa and Puma) [3]. The interactions between the antiapoptotic and the proapoptotic proteins can alter the permeability of mitochondrial membrane and trigger the release of cytochrome c (Cyt c) or cause cascade activation of the caspases.

Tumor necrosis factor-α (TNFα) elicits a wide range of biological responses, which are involved in the regulation of cell death and participate in the governing of immune homeostasis [4]. TNFα mediates its biological effects through the activation of two distinct receptors, TNF receptor type I (TNFRI, p55) and TNF receptor type II (TNFRII, p75) [5]. Apoptosis is mainly induced through TNFR1 by two best characterized pathways. Studies of TNFα-induced apoptosis have demonstrated that after binding to TNFR1, TNFα activated a death-inducing signaling complex that subsequently activated caspase-8 [6–8]. The activated caspase-8 can directly activate caspase-3 or indirectly activate caspase-3 through a mitochondrial apoptotic pathway that leads to the release of Cyt c from mitochondria following the loss of mitochondrial transmembrane potential [9]. Furthermore, recent studies have shown that the c-Jun N-terminal kinase (JNK) is also involved in the apoptosis induced by TNFα [10–12]. Our previous report has shown that TNFα induced

apoptosis through JNK/Bax-dependent pathway in differentiated PC12 cells [13].

Bim is a BH3-only proapoptotic protein and a member of the Bcl-2 family with three major forms generated by alternative splicing: Bim_{EL}, Bim_L, Bim_S [14]. Some reports suggest that JNK induces apoptosis by directly phosphorylating Bim_{EL} and Bim_L [15–19]. Bim_{EL} and Bim_L are expressed in a variety of tissues and cell types, their activities are regulated posttranslationally by sequestration to the microtubular dynein motor complex through the interaction with the dynein light chain 1 (LC8) [20]. The phosphorylation by JNK disrupts the function of LC8 binding motif and consequently causes the release of Bim from the sequestration of dynein motor complexes, then engages the mitochondrial apoptotic pathway [15, 16].

The Bim clearly acts upstream of Bax and Bak, because it cannot induce apoptosis in cells lacking both Bax and Bak [21, 22]. How it induces the activation of Bax and Bak is addressed by two distinct models. The direct activation model holds that Bim can bind to Bax or Bak directly and promote their activation [3, 23, 24]. The indirect activation model suggests that Bim only binds to the antiapoptotic proteins and acts by displacing the Bax from the bindings of antiapoptotic proteins, which promotes Bax activation [25, 26]. In this model, Bim is a potent inducer of apoptosis simply because it can bind all the antiapoptotic proteins. The debate over the mechanism of how Bim induces Bax activation continues.

Fluorescence resonance energy transfer (FRET) is a nonradiative transfer of energy between 2 fluorophores that are placed in close vicinity and in a proper relative angular orientation [27, 28]. It is a powerful technique that can provide an insight into the spatial and temporal cell events in physiological condition in single living cell. It has been utilized to reveal the dynamic activity of enzyme, and it provides a view of protein location, protein translocation, small ligand binding, protein–protein interaction, conformational change and posttranslational modification in real-time [29–31]. This cannot be fully elucidated by traditional biophysical or biochemical approaches, which can only measure the average behavior of cell populations and the static spatial information available from fixed cells and thus cannot provide direct access to cells life event in their natural environment.

Given these backgrounds, it is important to determine the mechanism of how Bim induces Bax activation. In this study, combined with confocal fluorescence imaging, FRET and co-immunoprecipitation techniques, the role of Bim_L in Bax activation was investigated during TNFα-induced apoptosis in differentiated PC12 and MCF7 cells.

Our results indicate that the contribution of Bim_L to TNFα-induced apoptosis is significant and Bim_L displaces Bax from Bcl-x_L in the mitochondrion.

Materials and methods

Plasmid construction

Bim_L cDNA was cloned from rat pheochromocytoma cell line (PC12) cells total RNA by RT-PCR. The Bim_L cDNA was amplified with Pfu polymerase using the special primers introducing NheI site and BamHI site, and then confirmed by sequencing. The special primers were designed to remove the stop codon of the Bim_L gene. The special primers: UP 5′-ACACGCTAGCTATGGCCAAGC AACCTTCTGA-3′, DW 5′-ACACGGATCCACATGCCT TCTCCATACCAGAC-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 [10], E-GFP-Cyt-c was presented by Dr. G. J. Gores [32], YFP-Bax and YFP-Bcl-x_L were presented by Dr. A. P. Gilmore [33].

Cell culture, transfection and treatment

Naïve PC12 cells were cultured in a humidified (5% CO^2, 37°C) incubator in Dulbecco's modified Eagle medium (DMEM) culture medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), 5% horse serum (Hyclone, Logan, Utah, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml). Human breast adenocarcinoma cell line (MCF7) cells were cultured in DMEM supplemented with 10% FCS, penicillin and streptomycin. To obtain neuronally differentiated PC12 cultures, subconfluent cells were differentiated for up to 3 days in DMEM with 0.5% FCS and NGF (100 ng/ml) (R&D Systems, MN, USA). To ensure maximum bioavailability, NGF and serum were replaced every other day, and cells were plated at a various densities according to each experimental protocol.

Naïve PC12 cells and MCF7 cells were transfected with different plasmids by using the LipofectamineTM 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The solution of LipofectamineTM 2000 reagent was replaced with fresh culture medium after 5 h. MCF7 cells were examined after transfection for 48 h. Naïve PC12 cells were differentiated in an especial dish for another 3 days before examination.

CHX is a protein synthesis inhibitor that can enhance the effect of TNFα-induced apoptosis. The concentrations of TNFα plus CHX used in our experiments were 40 ng/ml and 15 μg/ml. All the chemicals kept in the medium throughout the experiments.

YFP-Bax and GPF-Bim_L translocation assays

To observe the translocation of Bax, differentiated PC12 and MCF7 cells were co-transfected with YFP-Bax and

DsRed-Mit. Then, the cells were treated with TNFα/CHX. A laser scanning microscope (LSM 510/ConfoCor 2) combination system (Zeiss, Jena, Germany) equipped with a Plan-Neofluar 40×/1.3 NA Oil DIC objective was used in this experiment. Confocal images of YFP-Bax fluorescence from the cells were obtained using a 488 nm excitation light from an argon laser and a 500–530 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. Cells were maintained at 37°C using the temperature regulator (Tempcontrol 37–2 digital, Zeiss) in the whole experiment process. Temperature elevations of culture liquid were less than 0.2°C throughout the experimental process. To observe the translocation of Bim_L, differentiated PC12 and MCF7 cells were co-transfected with GFP-Bim_L and DsRed-Mit. Then, the cells were treated with TNFα/CHX. Confocal images of GFP-Bim_L fluorescence from the cells were obtained using a 488 nm excitation light from an argon laser and a 500–550 nm band-pass filter.

Detection of Cyt c release

Differentiated PC12 and MCF7 cells were co-transfected with DsRed-Mit and E-GFP-Cyt-c. Before detection, cells were treated by TNF-α/CHX or plus JNK inhibitor SP600125 (20 μM) (Sigma Chemical Co., St. Louis, MO, USA) then imaged by the Zeiss LSM 510 confocal microscope at 37°C. The images of GFP-Cyt-c and DsRed-Mit were obtained separately and then merged. The Cyt-c released from mitochondria was determined based on the overlap of GFP-Cyt-c and DsRed-Mit fluorescence images.

FRET image acquisition and data analysis

To investigate the interaction between Bim_L and Bcl-x_L in living differentiated PC12 and MCF7 cells after TNFα treatment, the cells were co-transfected with GFP-Bim_L and YFP-Bcl-x_L. Then, the differentiated PC12 and MCF7 cells were treated with TNFα/CHX, and observed using LSM 510 confocal microscope. Upon Bim interacting with Bcl-x_L, GFP is in close proximity to YFP, the FRET efficiency between GFP and YFP would increase (Fig. 1). To measure the FRET effect, cells were excited at 458 nm with an Ar-ion laser. The emission fluorescence was split by a second dichroic mirror (secondary beam splitter NFT545) into two separate channels: the 500–550 nm band-pass (GFP channel) and the 550–615 nm band-pass (FRET channel). Digital image analysis for quantitative evaluation was performed using Zeiss Rel3.2 image processing software (Zeiss, Jena, Germany). In the case of any fluorescent proteins, after choosing the area of interest from the cell images, the average fluorescence intensities of FRET and GFP channels and the background were obtained. The background-subtracted fluorescence intensity of FRET divided by background-subtracted fluorescence intensity of GFP is the ratio of FRET/GFP. For direct comparisons, the FRET/GFP ratio at the first time point was normalized to 1.

Cells expressing only GFP or YFP were used to determine the parameters for the calculation. GFP and YFP were chosen as a FRET pair instead of the more widely used CFP and YFP pair, since GFP is significantly more photostable than CFP, and the emission spectrum of GFP has a considerable overlap to the excitation spectrum of YFP. These features increase the likelihood of detecting the FRET signal [34].

Co-immunoprecipitation

Cells were treated with TNFα/CHX for 12 h and lysed in a buffer containing 50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% TritonX-100, 100 μg/ml PMSF and Protease Inhibitor Cocktail Set I (Calbiochem, San Diego, CA).

Equal amounts of protein were precleared with protein A/G beads (Amersham) and incubated with the Bim_L or Bcl-x_L antibody for 2 h at room temperature. Then the Ab complexes were captured with protein A/G beads for 1 h at room temperature. After washing three times with the same lysis buffer, the beads were resuspended in the sample buffer, separated by SDS/PAGE and transferred to a PVDF membrane (Roche Applied Science, Mannheim, Germany).

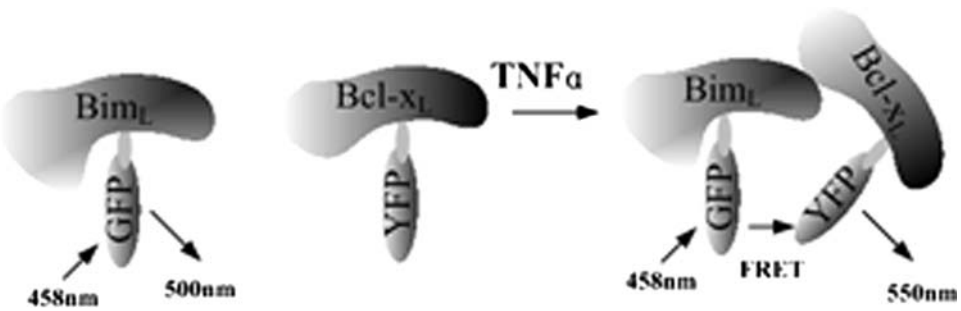

Fig. 1 Schematic diagram of the interaction between GFP-Bim_L and YFP-Bcl-x_L during TNFα-induced apoptosis

The membrane was first blocked with 5% skim milk for 1 h, followed by incubation with Bcl-x_L or Bax antibodies overnight at 4°C. All antibodies (Cell Signaling Technology, MA) were used at 1:1,000 dilutions. Antibody incubation was performed in 5% bovine serum albumin in TBS-T (Tris base saline, pH 7.4, 0.1% Tween 20). A designated secondary antibody diluted at the recommended ratio, in TBS-T and 5% bovine serum albumin for 1 h at room temperature. Washing with TBS-T was performed between all steps. The signals were detected by use of ODYSSEY Infrared Imaging System (Li-Cor, NE, USA).

Results

Bax translocation to mitochondria during TNFα-induced apoptosis

The dynamic translocations of Bax to mitochondria during TNFα-induced apoptosis in living differentiated PC12 and MCF7 cells were investigated by using confocal microscope. The cells were co-expressed with YFP-Bax and DsRed-Mit. YFP-Bax fusion protein was utilized to follow Bax migration and DsRed-Mit was used to label the mitochondria.

The YFP-Bax was distributed throughout the cytosol in the nonapoptotic differentiated PC12 and MCF7 cells (Fig. 2a). YFP-Bax translocated to the mitochondria markedly in differentiated PC12 and MCF7 cells, after TNFα/CHX treatment for 5 h 15 min and 5 h 30 min, respectively, as confirmed by the overlay images (Fig. 2b and c). The results suggest that TNFα induces apoptosis through Bax-dependent (or mitochondrial) pathway.

Detection of Cyt c release during TNFα-induced apoptosis

To further determine the apoptosis pathway, we examined the release of Cyt c from mitochondria into the cytosol in single living differentiated PC12 and MCF7 cells co-expressed with DsRed-Mit and E-GFP-Cyt-c during TNFα-induced apoptosis. Using confocal microscope and subcellular fractionation methods, the pattern of Cyt c-GFP fluorescence was found that it is indistinguishable from that of DsRed-Mit initially as shown in the control differentiated PC12 cells. These confocal images demonstrated that the

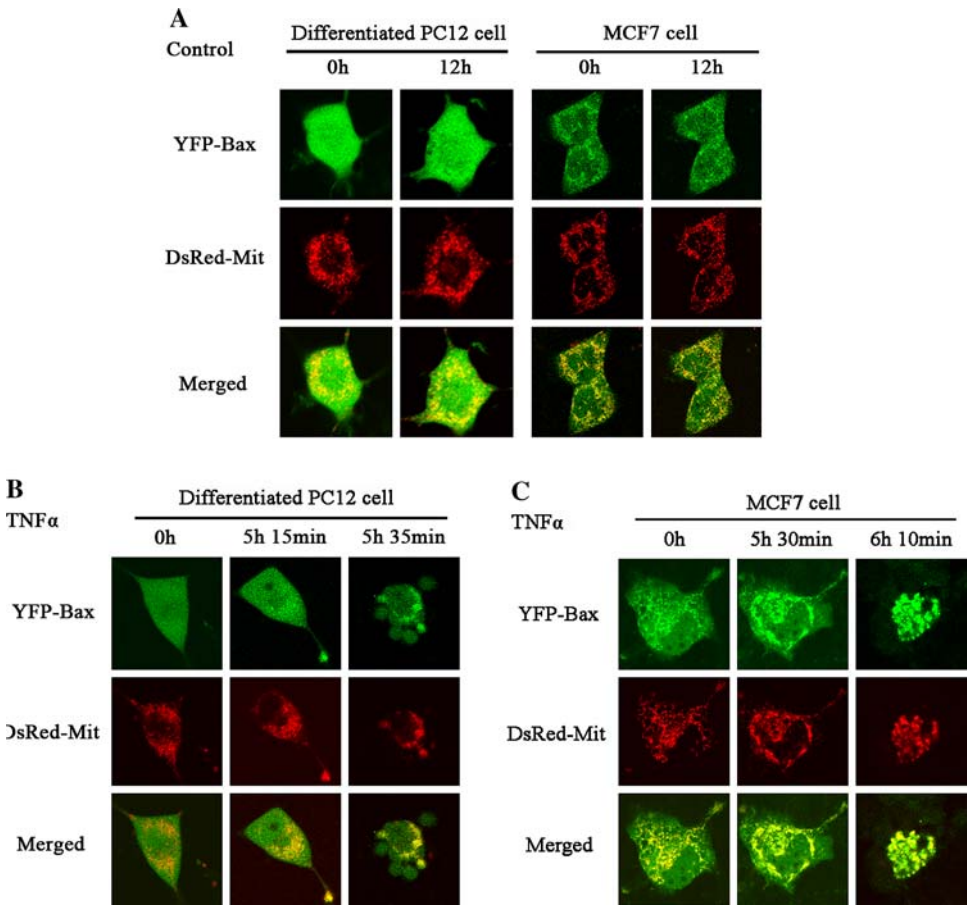

Fig. 2 Translocation of YFP-Bax treated with TNFα/CHX. Differentiated PC12 and MCF7 cells transiently co-expressing YFP-Bax and DsRed-Mit. Translocation of YFP-Bax was performed by laser fluorescence confocal microscopy. (**a**) Control cells without YFP-Bax translocation over time. (**b**) Differentiated PC12 cells treated with TNFα/CHX for 5 h 15 min, YFP-Bax translocated to mitochondria noticeably and apoptosis after 20 min. (**c**) MCF7 cells treated with TNFα/CHX for 5 h 30 min, YFP-Bax translocated to mitochondria noticeably and apoptosis after 40 min. Data are representative of three independent experiments

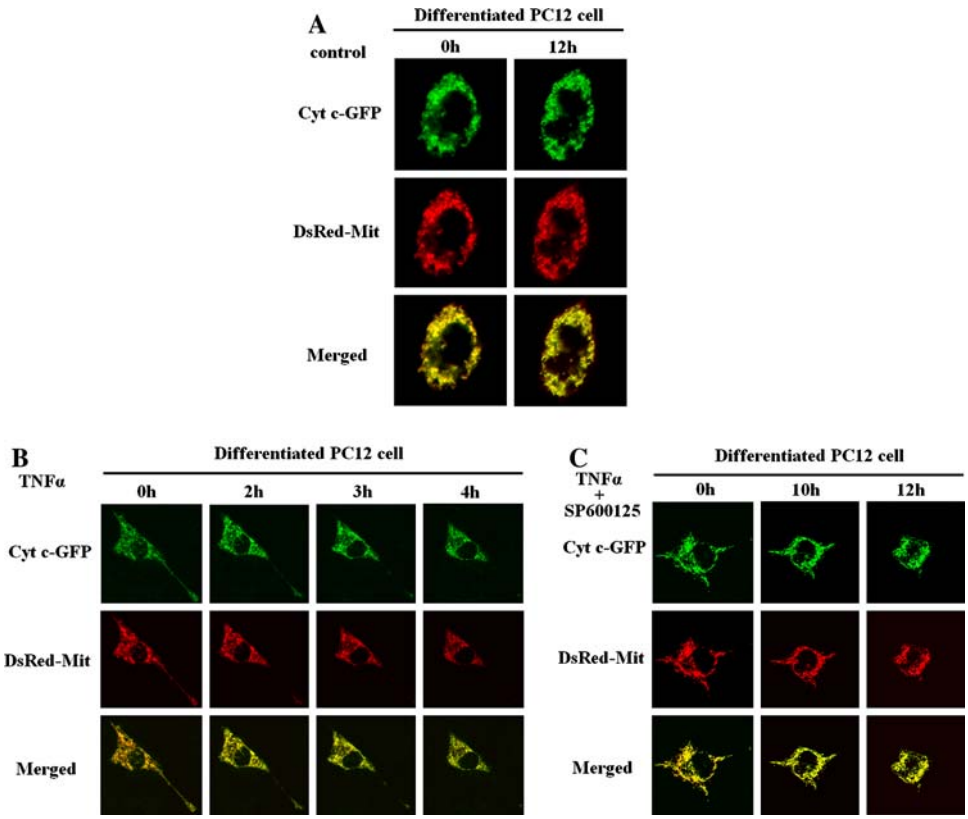

Fig. 3 Dynamics of Cyt c release in differentiated PC12 cells, co-expressing E-GFP-Cyt-c and DsRed-Mit, treated with TNF-α/CHX. (**a**) Control cells without Cyt c release over time. (**b**) Cells treated with TNFα/CHX for 4 h, Cyt c release from mitochondria noticeably. (**c**) Cells treated with SP600125 before TNF-α/CHX treatment, Cyt c did not release from mitochondria until the cell apoptosis. Data are representative of three independent experiments

distribution of Cyt c-GFP fusion protein was in the mitochondria (Fig. 3a). After the TNFα/CHX treatment, the pattern of Cyt c-GFP fluorescence in differentiated PC12 cells began to change from punctate to diffuse, indicating release of Cyt c from the mitochondria (Fig. 3b). In this process, Cyt c-GFP fluorescence was not fully diffuse, and some punctate Cyt c-GFP remained in mitochondria. However, SP600125 (specific inhibitor of JNK) could inhibit the release of Cyt c implying that TNFα induces apoptosis through mitochondrial pathway is JNK-dependent (Fig. 3c). The same results obtained in MCF7 cells (data not shown).

Bim_L directly interaction with Bcl-x_L during TNFα-induced apoptosis

Bim_L has been identified as an apoptosis inducer and it could be activated through phosphorylation by JNK. SP600125 inhibited the release of Cyt c during TNFα-induced apoptosis suggested that Bim_L was involved in this process. To determine whether Bim_L has effect, the interactions between Bim_L and Bcl-x_L, Bim_L and Bax, Bax and Bcl-x_L were tested by using co-immunoprecipitation. From Fig. 4, we found that Bcl-x_L co-immunoprecipitated with Bim_L increased after the TNFα treatment both in differentiated PC12 and MCF7 cells. In contrast, Bcl-x_L co-immunoprecipitated with Bax decreased. Moreover, Bax did not co-immunoprecipitated with Bim_L before or

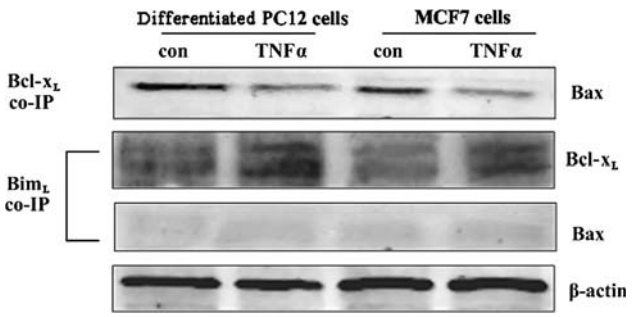

Fig. 4 Interactions between Bim_L and Bcl-x_L, Bim_L and Bax, Bim_L and Bcl-x_L after TNFα treatment. Differentiated PC12 and MCF7 cells were incubated with TNFα/CHX for 7 h, then the cell lysates were prepared and co-immunoprecipitation performed to analyze the interactions. Data are representative of three independent experiments

after the TNFα treatment. The result of the interaction between Bim_L and Bcl-x_L clearly demonstrates that Bim_L plays a role in the Bax translation during TNFα-induced apoptosis. Furthermore, all co-immunoprecipitation results suggest that Bim_L displaces Bax from Bcl-x_L after TNFα treatment.

Dynamic interaction between Bim_L and Bcl-x_L during TNFα-induced apoptosis

To further confirm the above results, the dynamic interaction between Bim_L and Bcl-x_L was performed in living

single-cell using FRET technique. Differentiated PC12 and MCF7 cells were co-transfected with GFP-Bim_L and YFP-Bcl-x_L, and then treated with TNFα/CHX to induce apoptosis. The typical time-course images of FRET, GFP and ratio of FRET/GFP in MCF7 cell after TNFα treatment are shown in Fig. 5a. The fluorescence images show that the emission in the FRET channel and the ratio of FRET/GFP channel increased, and the emission in the GFP channel decreased. All the ratios of FRET/GFP emission are confirmed by quantitative analysis of fluorescence intensities in the selected region (Fig. 5b). These results consist with that of co-immunoprecipitation experiment and further indicate that Bim_L displacing Bcl-x_L promotes Bax translocation during TNFα-induced apoptosis.

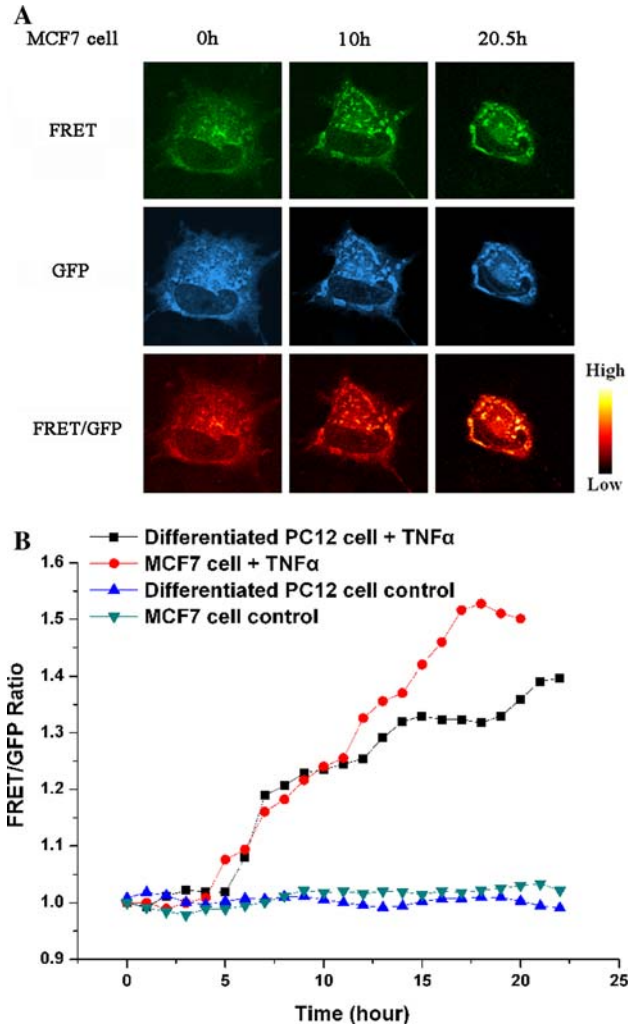

Fig. 5 Dynamic interaction between Bim_L and Bcl-x_L after TNFα treatment. Differentiated PC12 and MCF7 cells were co-expressing with YFP-Bcl-x_L and GFP-Bim_L. Cells were treated with TNFα/CHX after transfection and imaged by confocal microscopy. (**a**) Bcl-x_L and Bim_L distribution were imaged simulataneously in a MCF7 single cell. The images of FRET/GFP ratio were recorded with LSM microscope and processed with pseudocolor technique. (**b**) Dynamics of YFP/GFP ratio correspond to the images of YFP/GFP ratio. Similar results were obtained from three separate experiments

Bim_L not translocation to mitochondria during TNFα-induced apoptosis

To further determine the way of Bim_L inducing Bax activation, we examined the translocation of Bim_L in living differentiated PC12 and MCF7 cells. The cells were co-expressed with DsRed-Mit and GFP-Bim_L. Typical images of real-time distribution of GFP-Bim_L in control nonapoptotic cells are shown in Fig. 6a. Confocal microscopy images reveal that Bim_L localized largely in the intracytoplasmic membranes. After TNFα treatment, there was no evidence of Bim_L redistribution to mitochondria until cell apoptosis (Fig. 6b and c). In order to demonstrate the translocations of Bax and Bim_L more clearly, detailed time courses of the mitochondrial YFP-Bax and GFP-Bim_L fluorescence intensity were shown in Fig. 7. These results further confirmed that Bim_L activate Bax indirectly and Bim_L displaces Bax from Bcl-x_L in the mitochondrion.

Discussion

To our best knowledge, the molecular mechanism of Bax activation during TNFα-induced apoptosis was investigated for the first time by using the real-time single-cell analysis.

Bax is an executor of the mitochondrial pathway of apoptosis whose activation can be prevented by antiapoptotic Bcl-2 family proteins. Bax translocation from cytosol to mitochondria is a critical step in many drug-mediated apoptosis [33, 35]. In many cells, Bax locates predominantly in the cytosol, but translocates to mitochondria at an early stage in apoptosis [36–38]. In our experiments, Bax also translocates to mitochondria markedly in differentiated PC12 and MCF7 cells after TNFα/CHX treatment (Fig. 2b and c). To further determine the apoptosis pathway, we investigated the release of Cyt c from mitochondria. Cyt c released from mitochondria to cytosol during TNFα-induced apoptosis was observed (Fig. 3b). Further experiments provided evidence that SP600125 as an inhibitor of JNK could inhibit the release of Cyt c and reduce the TNFα-induced apoptosis (Fig. 3c). These results prove that TNFα induces apoptosis through the mitochondria pathway which is JNK-dependent.

What is the molecular mechanism of Bax activation? BH3-only proteins have been shown to be upstream regulators of Bax activation [15]. However, the mechanisms of how BH3-only proteins regulate the activity of Bax remain uncertain. Our previous report has shown that TNFα-induced apoptosis involved Bim in differentiated PC12 cells [37].

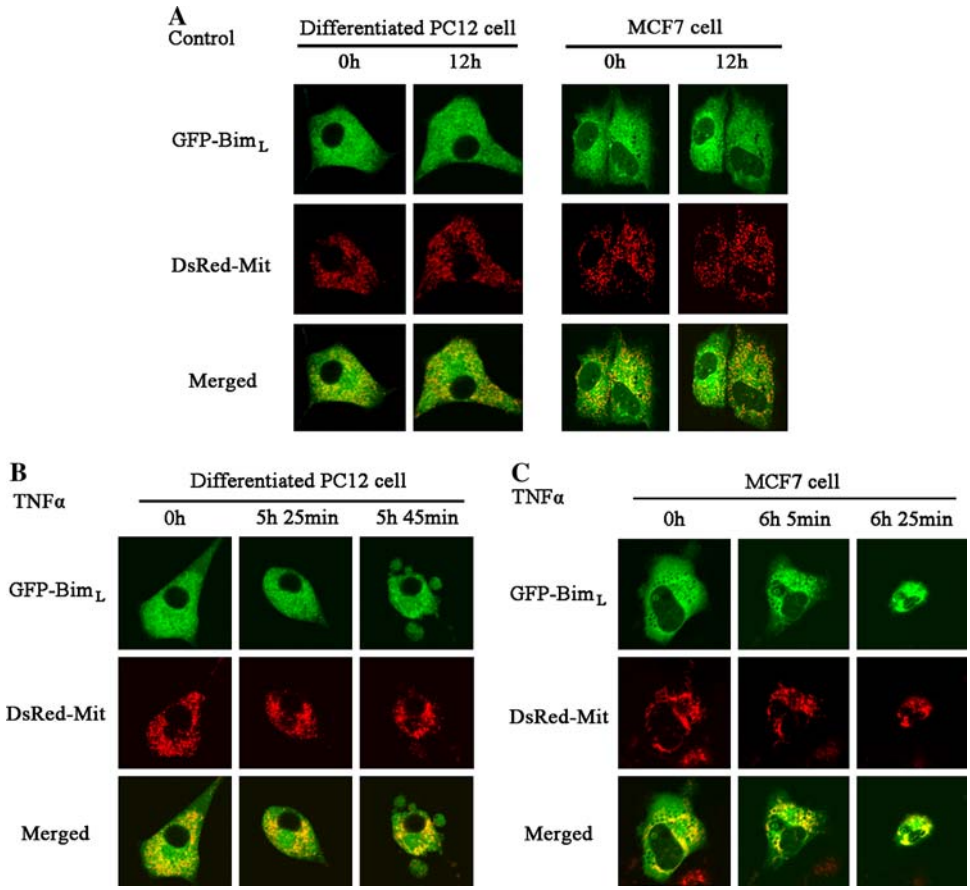

Fig. 6 Translocation of GFP-Bim$_L$ treated with TNFα/CHX. Differentiated PC12 and MCF7 cells transiently co-expressing GFP-Bim$_L$ and DsRed-Mit. Translocation of GFP-Bim$_L$ was performed by laser fluorescence confocal microscopy. (**a**) Control cells without GFP-Bim$_L$ translocation over time. (**b**) Differentiated PC12 cells and (**c**) MCF7 cells had no evidence of Bim$_L$ redistribution to mitochondria until cell apoptosis. Data are representative of three independent experiments

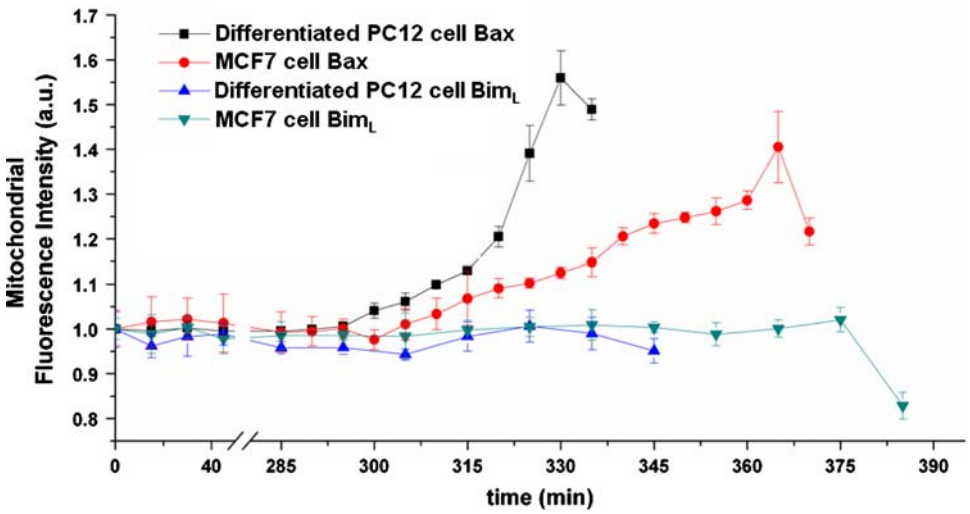

Fig. 7 Time courses of the mitochondrial YFP-Bax and GFP-Bim$_L$ fluorescence intensity. The mitochondrial YFP-Bax and GFP-Bim$_L$ fluorescence intensity during treatment comes from the overlay image shown in (Figs. 2 and 6)

In this study, the co-immunoprecipitation results demonstrated that Bim$_L$ interacted directly with Bcl-x$_L$ but not with Bax (Fig. 4). To further confirm the result, the dynamic interaction between Bim$_L$ and Bcl-x$_L$ was monitored in living single cells using FRET technique. The result consists with that of the co-immunoprecipitation experiment and further confirms that Bim$_L$ indeed interacts with Bcl-x$_L$ directly. Moreover, the apoptotic time can be delay to 20 h both in the differentiated PC12 and MCF7 cells, which cells co-transfected with GFP-Bim$_L$ and YFP-Bcl-x$_L$ (Fig. 5). The results also demonstrate that Bcl-x$_L$ negatively regulates apoptosis.

The results of Bim$_L$ translocation by the confocal microscope showed that Bim$_L$ had no obvious redistribution associated with mitochondria until cell apoptosis (Fig. 6b and c). These results suggest that Bim$_L$ performs its function in the mitochondria during TNFα-induced apoptosis.

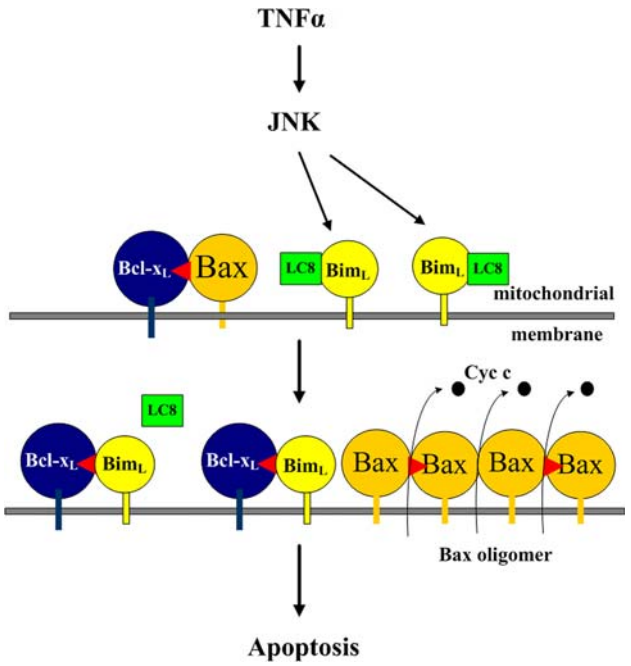

Fig. 8 A model of Bim_L displacing Bcl-x_L promotes Bax translocation during TNFα-induced apoptosis

Since Bim_L did not translocate to mitochondria, how it could induce Bax translocation? Firstly, many reports have documented that Bcl-x_L is located in the mitochondrial outer membrane and formed binding with Bax to inhibit apoptosis [1, 39, 40]. Secondly, in the indirect activation model of Bim_L activating Bax, Bim_L only binds to the antiapoptotic proteins and acts by displacing the Bax from the bindings of antiapoptotic proteins, and then promotes Bax activation [25, 26]. Finally, Bim_L possesses a hydrophobic C-terminus, localizing to intracytoplasmic membrane (including mitochondrial membrane) and sequestrated by LC8. Some reports showed that JNK mediated Bim_L phosphorylation could decrease the binding of Bim_L to LC8 [16, 41]. Moreover, phosphorylation of Bim_{EL} did not alter its subcellular localization or integration into mitochondrial membranes [15].

On the basis of previous reports and our findings, we speculated that phosphorylation of Bim_L could decrease the binding with LC8, and displace Bax from Bcl-x_L in the mitochondrion after TNFα treatment. Then, the free primed Bax could nucleate formation of the Bax oligomers that elicit permeabilization of the mitochondrial outer membrane (Fig. 8).

Conclusion

In this study, based on real-time single-cell analysis, the role of Bim_L in Bax activation was clearly elucidated during TNFα-induced apoptosis in differentiated PC12 and MCF7 cells. The experimental results strongly demonstrate that Bim_L involved in TNFα-induced apoptosis pathway and interacted directly with Bcl-x_L but had no interaction with Bax. In addition, Bax translocated to mitochondria but Bim_L not, indicating that Bim_L displacing Bcl-x_L promotes Bax translocation in the mitochondria during TNFα induced apoptosis.

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