Pathol. Oncol. Res. (2012) 18:809–816
DOI 10.1007/s12253-012-9508-x

RESEARCH

# Dihydroarteminsin-Induced Apoptosis is not Dependent on the Translocation of Bim to the Endoplasmic Reticulum in Human Lung Adenocarcinoma Cells

**Min Chen · Tong-sheng Chen · Ying-ying Lu · Cheng-yi Liu · Jun-le Qu**

Received: 16 May 2011 / Accepted: 16 February 2012 / Published online: 7 March 2012


**Abstract** Bim, a proapoptotic BH3-only member of Bcl-2 family, has been considered to play an important role in initiating mitochondrial apoptotic pathway. Our previous studies have shown the ability of dihydroarteminsin (DHA) to induce apoptosis in human lung adenocarcinoma (ASTC-a-1) cells. In this study, we investigated the function of Bim during DHA-induced apoptosis in ASTC-a-1 and another human lung adenocarcinoma (A549) cell lines. Confocal imaging of single living cell expressing GFP-BimL showed the translocation of Bim to endoplasmic reticulum (ER) rather than mitochondria during DHA-induced apoptosis. Moreover, we also found that DHA induced ER stress and an increase of Bim protein levels. However, silencing Bim by short hairpin RNA did not inhibit DHA-induced caspase-9 activation and cell apoptosis. Taken together, our results demonstrate for the first time that DHA induces Bim translocation to ER, but DHA-induced apoptosis is not dependent on Bim in ASTC-a-1 and A549 cell lines.

M. Chen · T.-s. Chen (✉) · Y.-y. Lu
MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, South China Normal University,
Guangzhou 510631, China
e-mail: chentsh@scnu.edu.cn

C.-y. Liu
Laboratory of Laser Sports Medicine, South China Normal University,
Guangzhou 510631, China

J.-l. Qu
Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education & Guangdong Province, Institute of Optoelectronics, Shenzhen University,
Shenzhen 518060, China

**Keywords** Bim · Dihydroartemisinin (DHA) · Endoplasmic reticulum (ER) · Apoptosis · Human lung adenocarcinoma cells

**Abbreviation**

| | |
|---|---|
| Bim | Bcl-2-interacting mediator of cell death |
| DHA | dihydroarteminsin |
| ASTC-a-1 | human lung adenocarcinoma cell |
| A549 | human lung adenocarcinoma cell |
| GFP | green fluorescent protein |
| RFP | red fluorescent protein |
| ER | endoplasmic reticulum |
| Bcl-xl | B-cell lymphoma-extra large |
| Bcl-2 | B-cell lymphoma 2 |
| CHOP | C/EBP homologous protein |
| UPR | unfolded protein response |
| PERK | pancreatic ER kinase (PKR)-like ER kinase |
| ATF6 | activating transcription factor 6 |
| IRE1 | inositol-requiring enzyme 1 |
| ROS | reactive oxygen species |
| NF-κB | nuclear factor-κB |
| DMSO | dimethyl sulphoxide |
| FRAP | fluorescence recovery after photobleaching |
| RNAi | RNA interference |
| shRNA | short hairpin RNA |
| shNC | short hairpin negative control |
| CCK-8 | cell counting kit-8 |
| OD | optical density |
| TBS-T | Tris base saline-Tween 20 |
| SD | standard deviation |
| FOXO3a | fork head boxo 3a |
| C2C12 | mouse myoblast cell line |
| BH3 | Bcl-2 homology domain 3 |

## Introduction

Bcl-2-interacting mediator of cell death (Bim), a proapoptotic BH3-only member of Bcl-2 family, is required for initiation of apoptosis induced by a broad range of cytotoxic stimuli [1]. Three Bim isoforms (BimEL, BimL, and BimS) have been identified [2]. Under physiologic conditions, Bim is bound to the dynein light chain (LC8) of the microtubular complex and strictly confined to cytosol [3]. It is generally considered that Bim participates in apoptosis in two ways: mitochondrial apoptotic and ER apoptotic pathways [4, 5]. Bim initiates the mitochondrial cell death pathway via either directly activating Bax/Bak protein (direct activation model) or indirectly activating Bax/Bak protein by binding to pro-survival Bcl-2 family members and thereby releasing Bax/Bak protein (indirect activation model) [6]. In addition, Bim has been shown to be involved in ER apoptotic pathway in which Bim translocates to ER and participates in ER stress-induced apoptosis, while stable overexpression of Bcl-xl suppresses the accumulation of Bim on ER and the apoptosis induced by ER stress [7]. Furthermore, Puthalakath et al [8] reported that Bim played a key role in ER stress-induced MCF-7 cell apoptosis, in which Bim was regulated by phosphatase protein phosphatase 2A and C/EBP homologous protein (CHOP).

ER stress is receiving increased attention because it is considered a cause of pathologically relevant apoptosis and is especially implicated in neurodegenerative disorders [9]. In higher eukaryotes, ER stress is elicited by the unfolded protein response (UPR) through three ER-resident transmembrane proteins: pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1) [5]. The initial combined activation of PERK, ATF6 and IRE1 promote the self protection measures taken by cells, however, when the ER stress is extensive or sustained, proapoptotic molecules such as CHOP and caspase-12 are activated to initiate the apoptotic response [8, 10].

Dihydroartemisinin (DHA), a first-line anti-malarial drug isolated from the traditional Chinese herb Artemisia annua [11], has been demonstrated to have anticancer effects [12–14]. The primary mechanism by which DHA exerts anticancer activity is thought to induce apoptosis [15]. It is believed that DHA exerts its cytotoxic effects by the generation of organic free radicals, resulting in induction of reactive oxygen species (ROS) from the iron-mediated cleavage of endoperoxide bridge contained in DHA [16]. DHA has been shown to induce apoptosis by inactivating nuclear factor-κB (NF-κB) and regulating its related gene products such as c-myc, Bcl-xl, Bcl-2 and Bax [14]. Moreover, it has been reported that DHA induces human hepatoma cells apoptosis via both p53-dependent and p53-independent pathways in vitro and in vivo [17]. We recently found that exposure of human lung adenocarcinoma (ASTC-a-1) cells to DHA induced ROS- and mitochondria-dependent apoptosis, in which Bax and Bid were found to translocate to mitochondria [12, 13]. The detailed function of Bim underlying DHA-induced ASTC-a-1 cell apoptosis remains unclear. The efforts of this report were directed toward examining the contribution of Bim to DHA-induced apoptosis in ASTC-a-1 and another human lung carcinoma (A549) cell lines.

## Materials and Methods

### Materials

DHA was obtained from Bide Pharmaceutical Corporation (Guangzhou, Guangdong Province, China). Working solutions were prepared by dissolving the compound in dimethyl sulphoxide (DMSO) before experiments. The final concentration of DMSO was less than 1% in all experiments. Mitotracker Red 633 was purchased from Invitrogen (Carlsbad, California). TurboFect in vitro anti-Bim, anti-β-actin, anti-caspase-9 antibodies were obtained from Cell Signaling (Beverly, Massachusetts). Anti-ATF-6, anti-P-PERK antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, California). All the secondary antibodies were supplied by Molecular Probes (Eugene, Oregon).

### Cell Culture and Transfection

ASTC-a-1 and A549 cell lines were obtained from the Department of Medicine, Jinan University (Guangzhou, China), and cultured in DMEM (GIBCO, Grand Island, New York) supplemented with 10% fetal calf serum (Sijiqing, Hangzhou, China) in 5% CO2 at 37°C in a humidified incubator. For fluorescence studies, cells were transiently transfected with plasmids using TurboFect in vitro Transfection Reagent (Fermentas, Vilnius, Lithuania) in a 35-mm dish for 24 to 48 h.

### Detection of Bim Translocation and Fluorescence Recovery After Photobleaching (FRAP) Analysis Inside Single Living Cell

A confocal laser scanning microscope (LSM510/ConfoCor2, Zeiss, Jena, Germany) was used to perform fluorescence imaging of Bim translocation and FRAP analysis inside single living cells. Images of cells co-expressing GFP-BimL and RFP-ER were collected using dual fluorescence channels. The excitation wavelengths were 488 nm for GFP and 543 nm for RFP and Mitotracker Red 633 (Invitrogen, Carlsbad, California). The emission fluorescence channels

were 500–550 nm for GFP and 600–650 nm for RFP and DsRed. For FRAP, the GFP in the indicating regions of living cells were photobleached using the maximal 488 nm laser line, and subsequent the entire cell was imaged at every 5 s with a low laser power (5% power) excitation for a duration of 600 s to monitor the recovery of fluorescence.

### RNA Interference (RNAi)

Bim suppression was accomplished using Bim shRNA (short hairpin RNA) constructs (GenePharma, Shanghai, China) as described previous [1]. The shRNA sequences were transfected into cells using TurboFect siRNA Transfection Reagent according to the manufacturer's protocol (Fermentas Vilnius, Lithuania).

### Cell Viability Assay

Cell viability was assessed by cell counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) as described previously [18, 19]. $OD_{450}$, the absorbance value at 450 nm, was read with a 96-well plate reader (DG5032; Huadong, Nanjing, China) to determine cell viability.

### Apoptosis Analysis

The proportion of cells in sub-G1 (apoptosis) phase was determined by FCM analysis of DNA content [12, 13]. To evaluate the cell cycle profile, cells were harvested, washed twice with PBS, and fixed in ice-cold 70% (v/v) ethanol for 1 h at 4°C. Prior to analysis, samples were washed again and incubated in PBS containing 10 μg/ml RNase A for 30 min, and then incubated with 5 μg/ml PI at 37°C in the dark for 30 min. DNA content was determined using a FCM (FACS, Arla BD, San Jose, California). For each analysis, 30,000 events were recorded.

### Western Blot Analysis

Preparation of whole cell lysates and western blot were carried out as previously described [12, 13]. Briefly, cells were lysed in a protein lysate buffer and centrifugated at 12,000×*g* for 10 min at 4°C. The samples containing 50 μg of protein were separated by SDS-PAGE and transferred to nitrocellulose membranes (Roch). The membranes were blocked with 5% skim milk, incubated with primary antibodies overnight, and subsequently with designated secondary antibody (Molecular Probes, Eugene, Oregon). Washing with TBS-T (Tris base saline, pH 7.4, 0.1% Tween 20) was performed between all steps. Detection was performed using the Odyssey Scanning Infrared Fluorescence Imaging System (LI-COR, Lincoln, Nebraska).

### Statistical Analysis

Results were expressed as mean ± standard deviation (SD) from at least three separate experiments, and significance was analyzed with student'st-test using the statistical software SPSS, version 10.0 (SPSS, Chicago). Difference were deemed to be statistically significant when $P<0.05$.

## Result

### DHA Induces Bim Translocation to ER and ER Stress in ASTC-a-1 Cells

In our previous studies [12, 13], we demonstrated that 20 μg/mL of DHA induced cell death of ASTC-a-1 cells in apoptotic fashion. In the present study, this concentration of DHA was used to treat cells. We used western blot analysis to examine the expression levels of Bim at 0, 12, 24 and 48 h after DHA treatment. As shown in Fig. 1a, DHA treatment induced a marked time-dependent increase in the protein levels of long and short forms of Bim (BimL and BimS).

Next, we use confocal fluorescence microscope to detect the spatial distribution of Bim in single living cells expressing GFP-BimL during DHA-induced apoptosis. Compared with control cells, DHA induced a significant translocation of Bim (Fig. 1b). Statistical results from 200 cells in three independent experiments showed a significant increase of the percentage of cells showing Bim translocation from 10.01% (0 h) to 36.35% (24 h) and 56.56% (48 h) after DHA treatment (Fig. 1c). To determine whether DHA induced Bim translocation to mitochondria, we monitored the dynamics of Bim translocation inside single living cells expressing GFP-BimL and stained with Mitotracker Red 633 using time-lapse confocal fluorescence microscope. It was disappointed that Bim did not translocate to mitochondria after DHA treatment (Fig. 1d). To further detect whether DHA induced Bim translocation to ER, we monitored the spatial distribution of Bim and ER in single living cells co-expressing GFP-BimL and RFP-ER. Surprisingly, the punctuated GFP-BimL co-localized with RFP-ER (Fig. 1e), indicating that Bim translocated to ER during DHA-induced cell apoptosis.

We also examined whether DHA triggered ER stress. Western blot analysis showed a time-dependent increase in ER stress related proteins ATF-6 and P-PERK after DHA treatment (Fig. 1f), suggesting that DHA triggered ER stress.

Collectively, these results revealed that DHA induced the increase of Bim expression, Bim translocation to ER and ER stress.

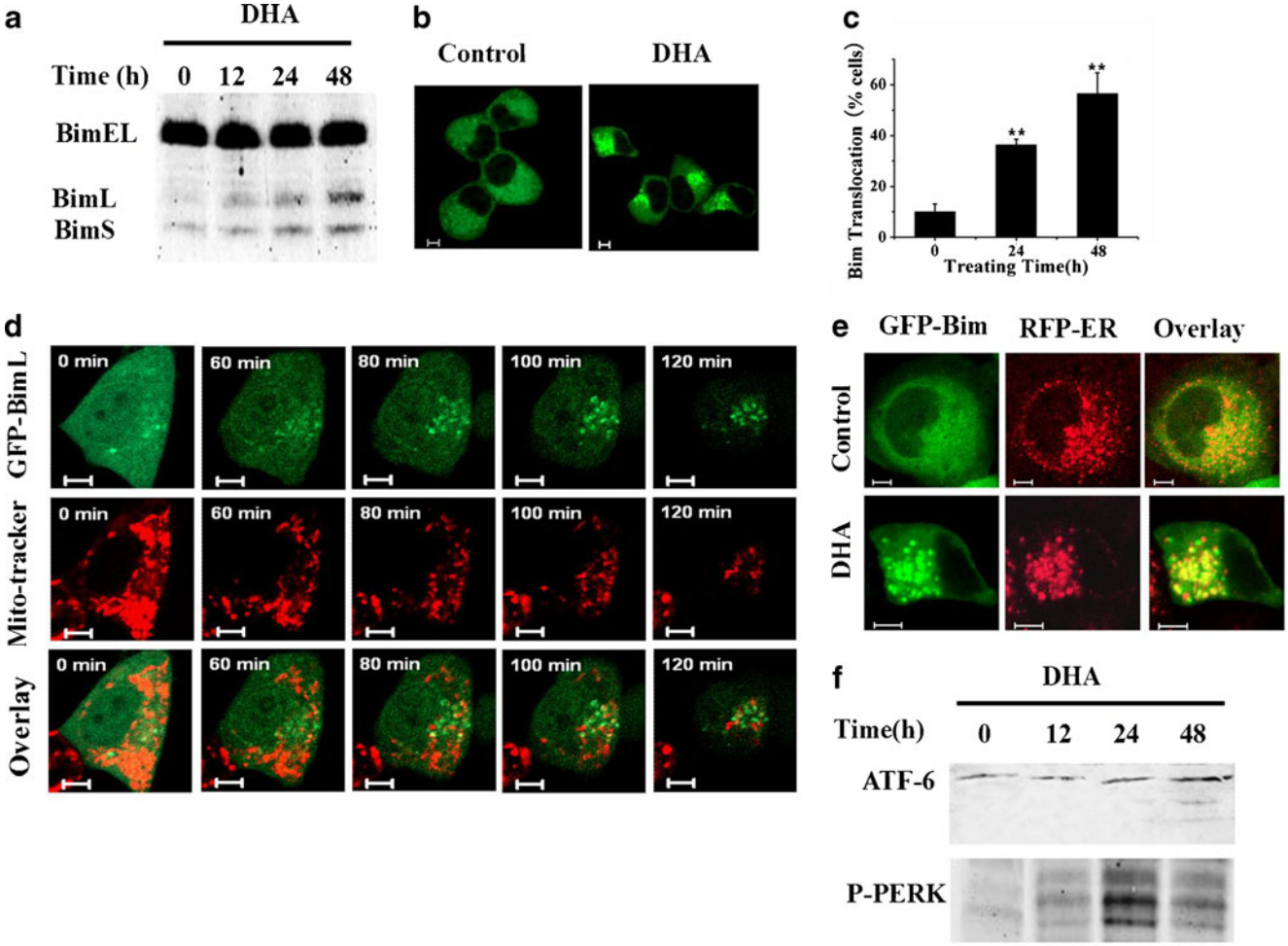


**Fig. 1** DHA induces the increase of Bim expression, Bim translocation to ER and ER stress in ASTC-a-1 cells. (**a**) Increase of Bim expression levels after DHA treatment for different time points (0, 12, 24, 48 h) assessed by western blot analysis. (**b**) Typical fluorescence images of living cells showing GFP-BimL distribution. Left panel: Control; Right panel: DHA-treated cells. Scale Bar: 5 μm. (**c**) Quantification of cells showing GFP-BimL translocation from 200 cells randomly selected from three independent experiments. Cells were treated with DHA for 0, 24 and 48 h. $^{**}P<0.01$, compared with control. (**d**) Dynamical fluorescence images of typical single living cell expressing GFP-BimL and stained with Mito-tracker. Scale Bar: 5 μm. (**e**) Typical fluorescence images of Bim translocation to ER inside single living cell after DHA treatment. Control cells show the uniform distribution of Bim, while DHA-treated cells show the co-localization between Bim and ER. Scale Bar: 5 μm. (**f**) Western blot analysis of the expression of ATF-6 and P-PERK after DHA treatment for different time points (0, 12, 24, 48 h)

## Bim is not Involved in DHA-Induced Apoptosis in ASTC-a-1 Cells

RNAi was used to examine the role of endogenous Bim in DHA-induced apoptosis. Western blot analysis showed that shBim resulted in a significant reduction of endogenous Bim protein compared to negative control shNC (Fig. 2a). As shown in Fig. 2b, DHA induced time-dependent caspase-9 activation, but silencing Bim by transfection of shBim failed to inhibit DHA-induced casapse-9 activation. Moreover, silencing Bim did not inhibit DHA-induced apoptosis at 24 and 48 h after DHA treatment (Fig. 2c and d), suggesting that Bim was not involved in DHA-induced cell apoptosis. Next, we used confocal microscopy to monitor the tempo-spatial distribution of FOXO3a, one of upstream transcription factors of Bim [20], inside single living cells expressing GFP-FOXO3a, and found that FOXO3a did not translocate to nucleus during DHA-induced apoptosis (Fig. 2e). Therefore, we make a conclusion that FOXO3a-Bim pathway was not involved in DHA-induced ASTC-a-1 cell apoptosis.

## Bim is not Involved in DHA-Induced Apoptosis in A549 Cells

We also examine the function of Bim in DHA-induced apoptosis of A549 cells. We used FRAP technique to assess

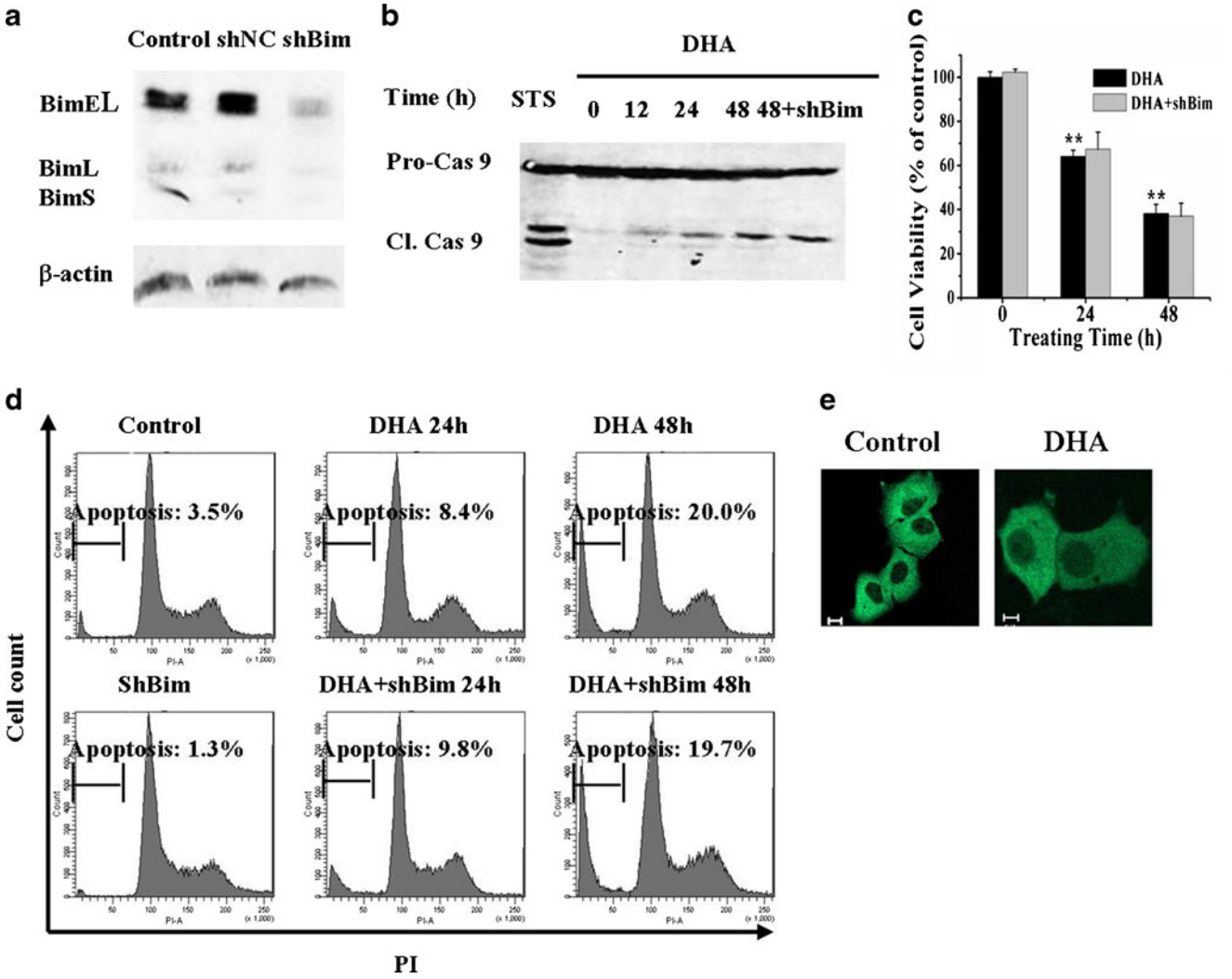


**Fig. 2** Knockdown of Bim does not inhibit DHA-induced activation of caspase-9 and apoptosis in ASTC-a-1 cells. **a** Down-regulation of Bim protein levels assessed by western blot. Cells were transfected with shBim and cultured for 36 h. shNC was a negative control for shRNA. β-actin served as a loading control. **b** Western blot analysis of the cleavage of caspase-9 with or without knockdown of Bim at different time points. **c** Down-regulation of Bim does not inhibit DHA-induced cell death. Cells viability was assessed by the CCK-8 assays. $^{**}P<0.01$, compared with control. **d** Down-regulation of Bim does not inhibit DHA-induced apoptosis. FCM was used to analysis Sub-G1 phase. Cells were cultured with 20 μg/ml DHA for 0, 24, or 48 h with or without infection of shBim plasmid and then stained with 5 μg/ml PI before being analyzed by FCM. **f** Typical fluorescence images of GFP-FOXO3a distribution in living cells. Left panel: Control; Right panel: DHA-treated cells. Scale Bar: 5 μm

the mobility of GFP-BimL fusion protein in single living cells. Figure 3a showed the typical fluorescence recovery images of the photobleaching area for control and DHA-treated cells, and the dynamics of FRAP from 15 to 20 cells in three independent experiments for control and DHA-treated cells were shown in Fig. 3b. We noticed a rapid refilling of GFP-BimL in the photobleached area for control cells, but in DHA-treated cells, the fluorescence recovery of GFP-BimL in the photobleached area was markedly blocked (Fig. 3a and b), implying that DHA induced the formation of Bim clusters. The statistical results of the percentage of cells showing Bim translocation from 200 cells in three independent experiments showed a significant increase of Bim translocation from 9.16% to 38.73% at 24 h and 53.44% at 48 h after DHA treatment (Fig. 3c). Furthermore, in accordance with ASTC-a-1 cells, we also found that DHA induced Bim translocation to ER rather than mitochondria in A549 cells (Fig. 3d). In addition, silencing Bim did not prevent DHA-induced apoptosis at 24 and 48 h after DHA treatment in A549 cells as well (Fig. 3e and f). These results also demonstrated that although DHA triggered the translocation of Bim to ER, however, DHA-induced apoptosis is independent from Bim.

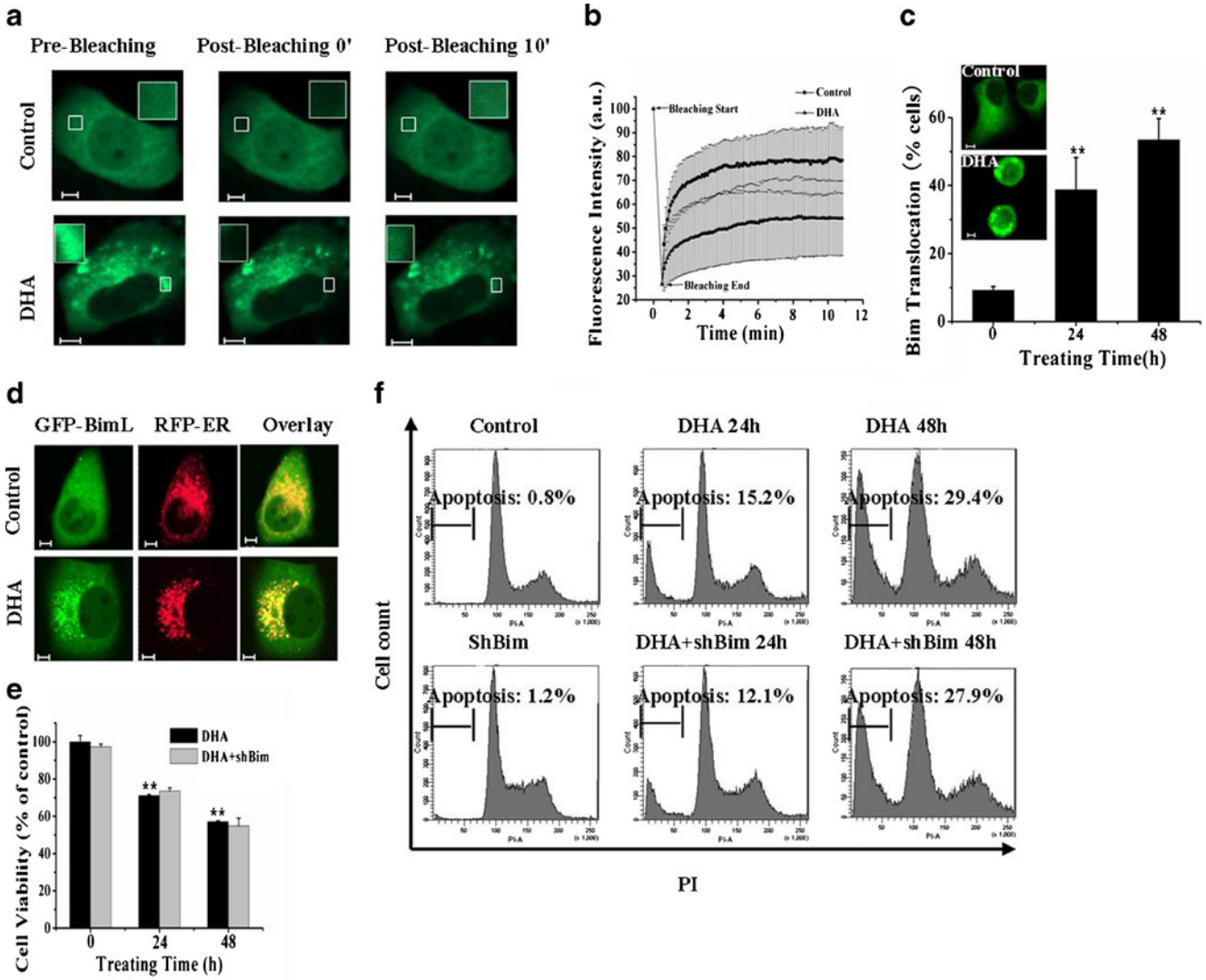


**Fig. 3** Not involvement of Bim in DHA-induced apoptosis of A549 cells. **a** Fluorescence recovery images of a typical living cell expressing GFP-BimL after photobleaching GFP. Upper panel: control cell; lower panel: DHA-treated cell. Scale Bar: 5 μm. **b** Dynamics of FRAP corresponding to (A) from 15 to 20 cells in three independent experiments for control, DHA-treated cells. Data were collected at 5 s intervals during recovery. **c** Quantification of cells showing GFP-BimL translocation from 200 cells randomly selected from three independent experiments for the indicated time. Cells were treated with DHA for 0, 24 and 48 h. $^{**}P<0.01$, compared with control. **d** Typical fluorescence images of Bim translocation to ER inside single living cell co-expressing GFP-BimL and RFP-ER. Cells were treated with 20 μg/mL of DHA for 24 h. Control cells show the uniform distribution of Bim, while DHA-treated cells show the co-localization between Bim and ER. Scale Bar: 5 μm. **e** Knockdown of Bim does not inhibit DHA-induced cell death assessed by the CCK-8 assays. $^{**}P<0.01$, compared with control. **f** Down-regulation of Bim does not inhibit DHA-induced apoptosis. FCM was used to analysis Sub-G1 phase. Cells were cultured with 20 μg/ml DHA for 0, 24, or 48 h with or without infection of shBim plasmid and then stained with 5 μg/ml PI before being analyzed by FCM

## Discussion

Our results provide the first evidence that Bim translocates to ER rather than mitochondria during DHA-induced apoptosis in human lung adenocarcinoma cells. Most studies have shown that Bim translocates to mitochondria to mediate the activation of Bax/Bak [1, 6]. However, Morishima and colleagues [7] recently reported that Bim translocated from cytoskeleton to ER in C2C12 cells exposed to tunicamycin. It is possible that Bim acts as a mediator between ER stress and proteins localized on the mitochondria membrane such as Bcl-xl [7]. In this study, we intuitively observe Bim translocation to ER in DHA-induced apoptosis. However, the underlying mechanisms of Bim translocation to ER remain unclear.

In addition to mitochondrial pathway, ER stress-induced ER pathway is also an important apoptotic signaling pathway [5, 10]. Different levels of ER stress trigger different physiological functions and cell reactions. ER stress response can promote cellular repair and sustain survival by

reducing the load of unfold proteins. When ER stress is persistent and overwhelming, apoptosis occurs to remove the damaged cells [8, 10]. Nakayama et al [21] recently demonstrated that lipopolysaccharide-induced ER stress did not induce apoptosis, but activated the ER function-protective pathway. However, tunicamycin and thapsigargin have been demonstrated to induce ER stress-mediated apoptosis by activating CHOP and caspase-12 in a number of cell lines [7]. The structure of DHA is similar to thapsigargin which is also a sesquiterpene lactone and acts as an ER stress inducer [9]. It has been reported that DHA causes iron-dependent ER stress in colorectal carcinoma HCT116 cells [22]. Our observation that DHA induces a modest increase of ATF-6 and P-PERK implicates that DHA treatment can induce a weak ER stress (Fig. 1f). Whether the ER stress mediates DHA-induced apoptosis need to be further explored.

It is reported that ER stress activates Bim to induce cell apoptosis by preventing its degradation and increasing its transcriptional induction [8]. Morishima et al [7] showed that ER stress triggered Bim accumulation on ER. Mohamed et al [23] reported that a broad range of cytotoxic stimuli such as thapsigargin can induce ER stress, and also increase the expression of Bim, suggesting that ER stress increases the expression levels of Bim. ER stress has also been found to increase the proapoptotic Bcl-2 proteins such as Bim and Puma in commitment phase [23, 24]. However, based on our results that ER stress was observed at 12 h after DHA treatment (Fig. 1f), while Bim translocation was found at 2 h (Fig. 1d), it is reasonable to speculate that Bim translocation to ER precedes ER stress during DHA-induced apoptosis.

Although DHA causes the increase of Bim protein levels and translocation of Bim to ER, the fact that silencing of Bim by RNAi failed to inhibit the DHA-induced casapse-9 activation (Fig. 2b) and cell apoptosis (Figs. 2c, d, 3e and f) demonstrate that Bim is not involved in DHA-induced apoptosis. It is demonstrated that BH3 proteins such as Bim are essential initiators of apoptosis, once activated, they regulate Bax or Bak to undergo a conformational switch and translocate to mitochondria or ER [25, 26]. Although DHA induced Bim translocation to ER (Figs. 1e and 3d), our previous studies showed that Bax translocated to mitochondria rather than ER during DHA-induced apoptosis in ASTC-a-1 [13] and A549 cells (data not shown). Therefore, we speculate that translocation of Bim to ER does not activate Bax/Bak localized on mitochondria to trigger apoptosis.

In conclusion, DHA treatment induces Bim translocation to ER, however, which is incapable of enhancing DHA-induced apoptosis in human lung adenocarcinoma cells. Therefore, we speculate that Bim translocation to ER is only an accompanying phenomenon in DHA-induced human lung adenocarcinoma cell apoptosis. This work will help improve our understanding about the translocation of Bim to ER and the molecular mechanisms of DHA-induced apoptosis.

**Acknowledgments** This study was supported by National Natural Science Foundation of China (Grant No. 31071218 and 61178078) and the Natural Science Foundation of Guangdong Province (Grant No. 9251063101000009).

## References

1. Wang XW, Xing D, Liu L et al (2009) BimL directly neutralizes Bcl-xL to promote Bax activation during UV-induced apoptosis. FEBS Lett 583:1873–1879
2. Yin KJ, Hsu CY, Hu XY et al (2006) Protein phosphatase 2A regulates bim expression via the Akt/FKHRL1 signaling pathway in amyloidpeptide-induced cerebrovascular endothelial cell death. J Neurosci 26:2290–2299
3. Schneiders UM, Schyschka L, Rudy A et al (2009) BH3-only proteins Mcl-1 and Bim as well as endonuclease G are targeted in spongistatin 1–induced apoptosis in breast cancer cells. Mol Cancer Ther 8:2914–2925
4. Khawaja NR, Carre M, Kovacic H et al (2008) Patupilone-induced apoptosis is mediated by mitochondrial reactive oxygen species through Bim relocalization to mitochondria. Mol Pharmacol 74:1072–1083
5. Szegezdi E, Logue SE, Gorman AM et al (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885
6. Mérino D, Giam M, Hughes PD et al (2009) The role of BH3-only protein Bim extends beyond inhibiting Bcl-2–like prosurvival proteins. J Cell Biol 186:355–362
7. Morishima N, Nakanishi K, Tsuchiya K et al (2004) Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signaling for activation of caspase-12 during ER stress-induced apoptosis. J Biol Chem 279:50375–50381
8. Puthalakath H, O'Reilly LA, Gunn P et al (2007) ER stress triggers apoptosis by activating BH3-only protein Bim. Cell 129:1337–1349
9. Chin TY, Lin HC, Kuo JP et al (2007) Dual effect of thapsigargin on cell death in porcine aortic smooth muscle cells. Am J Physiol-cell Ph 292:383–395
10. Morishima N, Nakanishi K, Takenouchi H et al (2002) An endoplasmic reticulum stress-specific caspase cascade in apoptosis. J Biol Chem 277:34287–34294
11. Dhingra V, Rao KV, Narasu ML (2000) Current status of artemisinin and its derivatives as antimalarial drugs. Life Sci 66:279–300
12. Lu YY, Chen TS, Wang XP et al (2010) Single-cell analysis of dihydroartemisinin-induced apoptosis through reactive oxygen species-mediated caspase-8 activation and mitochondrial pathway in ASTC-a-1 cells using fluorescence imaging techniques. J Biomed Opt 15:046028-1–16
13. Lu YY, Chen TS, Wang XP et al (2010) The JNK inhibitor SP600125 enhances dihydroartemisinin-induced apoptosis by accelerating Bax translocation into mitochondria in human lung adenocarcinoma cells. FEBS Lett 584:4019–4026
14. Wang SJ, GaoY CH et al (2010) Dihydroartemisinin inactivates NF-κB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both in vitro and in vivo. Cancer Lett 293:99–108

15. Handrick R, Ontikatze T, Bauer KD et al (2010) Dihydroartemisinin induces apoptosis by a Bak-dependent intrinsic pathway. Mol Cancer Ther 9:2497–2510
16. Mercer AE, Maggs JL, Sun XM et al (2007) Evidence for the involvement of carbon-centered radicals in the induction of apoptotic cell death by artemisinin compounds. J Biol Chem 282:9372–9382
17. Hou JM, Wang DS, Zhang RW et al (2008) Experimental therapy of hepatoma with artemisinin and its derivatives: in vitro and in vivo activity, chemosensitization, and mechanisms of action. Clin Cancer Res 14:5519–5530
18. Zhang YJ, Xing D, Liu L (2009) PUMA promotes Bax translocation by both directly interacting with Bax and by competitive binding to Bcl-XL during UV-induced apoptosis. Mol Biol Cell 20:3077–3087
19. Zhang WW, Wang ZP, Chen TS (2010) Curcumol induces apoptosis via caspases-independent mitochondrial pathway in human lung adenocarcinoma ASTC-a-1 cells. Med Oncol 28:307–314
20. Stan SD, Hahm ER, Warin R et al (2008) Withaferin A causes FOXO3a- and Bim-dependent apoptosis and inhibits growth of human breast cancer cells in vivo. Cancer Res 68:7661–7669
21. Nakayama Y, Endo M, Tsukano H et al (2010) Molecular mechanisms of the LPS-induced non-apoptotic ER stress-CHOP pathway. J Biochem 147:471–483
22. Lu JJ, Chen SM, Zhang XW et al (2010) The anti-cancer activity of dihydroartemisinin is associated with induction of iron-dependent endoplasmic reticulum stress in colorectal carcinoma HCT116 cells. Invest New Drug. doi:10.1007/s10637-010-9481-8
23. Mohamed R, Mayo M, Dash R et al (2010) Melanoma differentiation associated gene-7/interleukin-24 potently induces apoptosis in human myeloid leukemia cells through a process regulated by endoplasmic reticulum stress. Mol Pharmacol 78:1096–1104
24. Kim H, Tu HC, Ren D et al (2009) Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. Mol Cell 36:487–499
25. Gillissen B, Essmann F, Hemmati PG et al (2007) Mcl-1 determines the Bax dependency of Nbk/Bik-induced apoptosis. J Cell Biol 179:701–715
26. Zhang LL, Zhang YJ, Xing D (2010) LPLI inhibits apoptosis upstream of Bax translocation via a GSK-3β-inactivation mechanism. J Cell Physiol 224:218–228