PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2011, 38(9): 859~865
www.pibb.ac.cn

FHC Interacts With Bim and Protects Cell From Apoptosis*

ZOU Guan-Yu, JI Chao-Neng, SHEN Qi, XUE Jing-Lun, CHEN Jin-Zhong**
(*State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai* 200433, China)

Abstract It is known that both FHC and Bim is involved in the regulation of intracellular iron metabolism, and plays a role in cellular apoptosis caused by ROS. However, the molecular mechanisms of FHC regulating apoptosis are remaining unknown. Using pLexA-Bim L as bait, a pB42AD based cDNA library was screened and FHC was identified as a Bim interacting protein. The interaction domain on Bim was located to BH3 domain. The interaction between FHC and Bim was further verified by co-immunoprecipitation. The subcellular location assay revealed that both Bim and FHC are located to cytoplasm and partially overlap. Over expression of FHC in HEK293 protects the cells from cytotoxity caused by over expressing Bim L. Both over-expression and knock-down analysis of FHC suggest that FHC protects HEK293 cells from hydrogen peroxide treatment. A novel Bim interacting protein, FHC was identified and it was suggested that FHC play a role in Bim mediated apoptosis and oxidative stress.

Key words ferritin heavy chain, Bim, oxidative stress, apoptosis
DOI: 10.3724/SP.J.1206.2010.00608

Bcl-2 family proteins play a pivotal role in the regulation of mitochondrial integrity and in the mitochondrial apoptosis pathway [1]. There are two types of Bcl-2 proteins: anti-apoptotic Bcl-2 family proteins and pro-apoptotic members [2]. Since Bim S, Bim L and Bim EL were first identified in 1998, at least fifteen isoforms of Bim have been reported. The BH3 contained Bim isoforms present proapoptotic activity, while the isoforms without BH3 domain act as the decoys and present the anti-apoptotic activity. Abundant isoforms provide the possibility for Bim to play a role both as sensor and trigger in apoptotic cascade [3–7]. Bim L and Bim EL are the predominant isoforms and they have been shown to interact with all the survival members of Bcl-2 family. Bim was also reported to act as a direct activator of Bax and Bak，thus loss of Bim could confer protection from apoptosis. Vice versa, Bim cannot trigger apoptosis in mice lacking both Bax and Bak [8–11]. Bim is normally sequestered in cytosol by its association with the dynein light chain and is regulated at the transcriptional or post-transcriptional level by cytokines. The dynein light chain binding domain of Bim seems play a role in the regulation of Bim. Several signals such as TGF-beta, ROS and serum deprive result in Bim translocation from cytoskeleton to mitochondria [12–14]. Either the proapoptotic members of BCL2 protein family or the prosurvival members interacting with the BH3-only protein Bim results in initiating apoptosis [15]. Up to now, a role for Bim in regulating apoptosis is established, but it is incompletely understood [16]. To identify and characterize the Bim interacting proteins might enrich our knowledge of Bim mediated apoptosis.

It was reported that ROS regulate quiescent T-cell apoptosis *via* Bim [10, 17]. On the other hand, there seems another signaling pathway from ROS, FHC to apoptosis [18–19]. Accumulation of data suggested that the intracellular iron regulate both Bim and FHC, which might play a role in the cell survival or apoptosis [20]. These data raise an interesting question: Does the two

*This work was supported by grants from Hi-Tech Research and Development Program of China (2009ZX09503-20) and The Nature Science Foundation of China (30971617).

**Corresponding author.
Tel: 86-21-65642424, E-mail: kingbellchen@fudan.edu.cn

Received: February 28, 2011 Accepted: April 12, 2011

pathways cross-talk each other or they just work independently? Our experiments identified that FHC was a Bim interacting protein and FHC protected cells from apoptosis induced by over expressing Bim or oxidative stress.

1 Materials and methods

1.1 Plasmid construction

The pLexA-Bim bait plasmids for the yeast two hybrid assay and pEGFP- Bim plasmids for expressing EGFP-Bim fusion proteins were described previously[5, 7].

FHC cDNA was obtained from a pre-made human cDNA library of fetal brain (Clontech) by amplification with the following primers: 5′ GAGGAATTCCCATGACGACCGCGTCCACCTCGCAGG 3′; 5′ GAGCTCGAGGCTTTCATTATCACTGTCTCCCAGGGTGTG 3′. The cDNA was inserted into pCMV-Myc (Clontech) between *Eco*R Ⅰ and *Xho* Ⅰ to produce pCMV-Myc-FHC for over expressing FHC in cells. FHC cDNA was also amplified with primers (5′ GAGGAATTCACCATGACGACCGCGTCCACCTCGCAGG 3′; 5′ GAGGGATCCGCGCTTTCATTATCACTGTCTCCCAGGGTGTG 3′) and inserted into pDsRed2-N1 between *Hin*d Ⅲ and *Eco*R Ⅰ to generate pDsRed-FHC for subcellular location assay.

1.2 Cell culture

HEK293 cells (ATCC) are propagated in DMEM medium supplemented with 10% FBS in a humidified incubator under an atmosphere of 5% CO_2 at 37℃.

1.3 Yeast two-hybrid screening

The MATCHMAKER pLexA two-hybrid system was purchased from Clontech. The yeast two-hybridization was conducted with a standard two steps protocol (Clontech). Using pLexA-Bim L as bait, we screened the pre-made pB42AD-fetal brain cDNA library. All isolated positive clones were amplified with the uniform pB42AD sequence primers (5′ CCAGCCTCTTGCTGAGTGGAGATG 3′; 5′ GGAGACTTGACCAAACCTCTGGCG 3′) and the PCR products were sequenced with the same primers. Yeast mating test was used to determine on the interaction between FHC and Bim isoforms (Bims). pLexA-Bims were used to transfect YM4271 correspondingly, then mated with pB42AD-FHC contained EGY48[p8opLacZ].

1.4 Subcellular co-location assay

FHC-RFP and GFP-Bim L were co-expressed in the HEK293 cells through transiently co-transfection with pEGFP-Bim L and pDsRed-FHC using Lipofectamine(Invitrogen). 24 h after transfected, cells were fixed in 4% paraformaldehyde followed by staining with 4′, 6-diamidino-2-phenylindole (DAPI). The fluorescence of GFP, RFP and DAPI were observed under the fluorescent microscope (Olimpus).

1.5 Co-immunoprecipitation assay

HEK293 cells were growth in 6-well plates and were co-transfected with pCMV-Myc-FHC and pEGFP-Bims using Lipofectamine agent (Invitrogen). Cells co-transfected with pCMV-Myc-FHC and pEGFP-C1 were used as control. 24 h after transfected, the cells were harvested and lysed in 0.5 ml RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L PMSF, Aprotinin, leupeptin, pepstatin: 1 mg/L each, 1 mmol/L Na_3VO_4, 1 mmol/L NaF) for 15 min at 4℃. The lysates were centrifuged at 14 000 r/min for 5 min at 4℃ to remove cell debris for the further assay of co-immunoprecipitation. Mouse-anti-myc antibody (Santa Cruz) conjugated to protein A/G PLUS agarose beads (Santa Cruz) according to standard protocol recommended. After incubation with cells lysate for overnight at 4℃ with rotation, the protein A/G beads were collected by centrifugation at 500 *g* for 5 min and then washed 4 times with 1.0 ml RIPA buffer. The precipitates were resolved on 12% sodium dodecyl sulfate-containing polyacrylamide gels, and transferred to nitrocellulose membranes (Amersham). The blot was detected using rabbit anti-EGFP antibody (Santa Cruz) followed by horseradish-peroxidase conjugated anti rabbit IgG antibody (Sigma) and the bands were visualized using DAB substrates.

1.6 Knock down assay

Three FHC specific siRNA duplexes [FTH1-HSS177644：CUCUGAGUCCUGGUGGUAGUUCUGG, CCAGAACUACCACCAGGACUCAGAG (NM_002032, 265-289); FTH1-HSS177645: AUCGCGGUCAAAGUAGUAAGACAUG, CAUGUCUUACUACUUUGACCGCGAU (NM_002032, 346-370); FTH1-HSS177646: AGUUUGUGCAGUUCCAGUAGUGACU, AGUCACUACUGGAACUGCACAAACU (NM_002032, 573-597)] were purchased from Invitrogen and used to knockdown the expression of FHC. Also the expression of Bim was knocked down by three duplexes [BCL2L11-HSS145413: UUGUCUACCUUCUCGGUCACACUCA, UGAGUGUGACCGAGAAGGUAGACAA (NM_138621, 317-342); BCL2L11-HSS173328: UUGUGUUGAUUUGUCACAACUCA-

UG, CAUGAGUUGUGACAAAUCAACACAA(NM_138621, 609-633); BCL2L11-HSS173329: AACAGUCGUAAGAUAACCAUUCGUG, CACGAAUG - GUUAUCUUACGACUGUU(NM_138621, 824-848)] synthesized by Invitrogen. Cause the complexity of Bim isoforms, the GFP-Bim L expression plasmid and siRNA were co-transfected into the HEK273 cells, the expression of GFP-Bim L was assayed using Western blot with GFP specific antibody. As a negative control non-siRNA (UUCUCCGAACGUGUCACGU; ACGUGACACGUUCGGAGA) were used. The sample was detected by a GAPDH specific antibody as control.

1.7 Apoptosis assay

Apoptotic cells were quantified by flow cytometric analysis with APO-BRDU™ Apoptosis Assay Kit (Becton Dickinson) according to the manufacturer's recommendations. Each group repeated for three times.

To evaluate the protection of FHC to Bim induced apoptosis, the DBD contained isoforms of Bim L and the isofroms without DBD (Bim S) pEGFP-Bims plasmids were used to co-transfected HEK 293 with pCMV-myc-FHC. The pEGFP and pCMV-myc used as the mock. To evaluate the function of Bim and FHC on ROS stress, the FHC specific and a Bim specific duplex siRNA were used to knockdown the cellular FHC and Bims. 24 h after transfected, 20 μmol/L H_2O_2 was used as a ROS stimulating agent shield to the cell. The treatment maintained for 2 h, then replaced by fresh medium. The apoptosis was detected 24 h after H_2O_2 treatment.

2 Results

2.1 Isolation of FHC as a novel BimL-interacting protein

pLexA-Bim L bait plasmid was used to screen the pre-made pB42AD-human fetal brain cDNA library. 127 positive clones were isolated from 10^6 cDNA clones. Sequence analysis revealed that 23 clones of them encoded FHC. The interactions between FHC and Bim isoforms were revealed by yeast mating test. All detected isoforms interacted with FHC and suggested that BH3 domain was the interacting fragment (Figure 1).

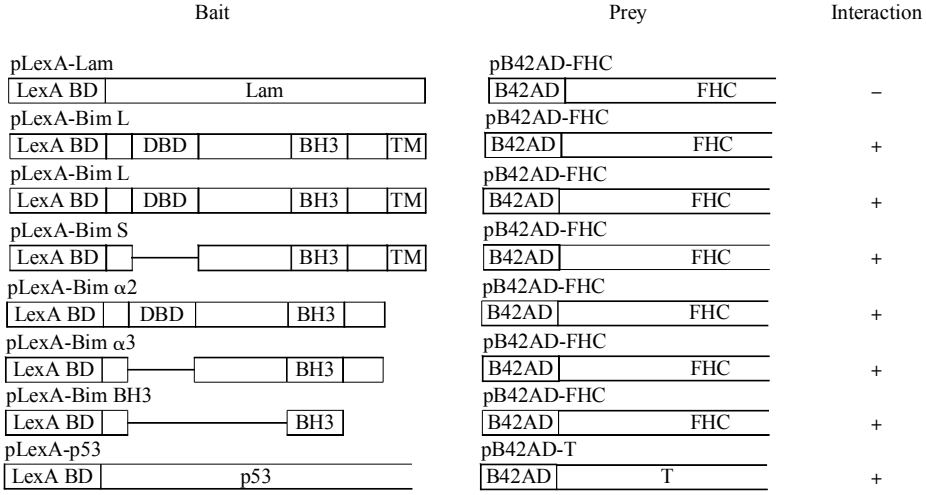

Fig. 1 The yeast-two-hybridization assay of FHC and Bim

pLexA- Bims plasmids contained YM4271 strains were mated with B42AD-FHC contained EGY48[p8opLacZ]. –: Present no interaction clones growth on the induce plate, +: Present the interacting blue clones growth on the induce plate. The Lam-FHC group is negative control. The positive control group is P53-large T interaction. BH3 refer to Bcl-2 homology domain 3; DBD refer to dynein binding domain; TM refer to transmembrane hydrophobic region.

pCMV-Myc-FHC and pEGFP-Bim L/pEGFP-Bim S co-transfected HEK293 cells were used for co-immunoprecipitation assay, pEGFP and pCMV-Myc-FHC co-transfected HEK293 cells were used as control. A mouse anti-myc epitope antibody precipitated GFP-Bims but not GFP from the cell lysates (Figure 2). The results of co-immunoprecipitation assay were consisted to the results of yeast-two-hybridization.

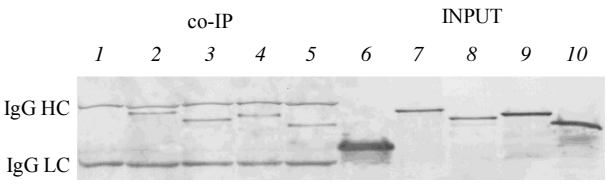

Fig. 2　Co-immunoprecipitation analysis of FHC and Bim in HEK293 cells

Samples were analyzed by Western blotting with a mouse anti-GFP antibody. Co-IP group: Cell lysates of pEGFP-C1/-Bims transfected HEK293 cells were incubated with a rabbit anti-FHC binding protein A/G agarose beads. INPUT group: Cell lysate of pEGFP-C1/-Bims transfected HEK293 cells. *1*: EGFP; *2*: EGFP-Bim L; *3*: EGFP-Bim S; *4*: EGFP-Bimα2; *5*: EGFP-Bimα3 ; *6*: EGFP; *7*: EGFP-BimL; *8*: EGFP-Bim S; *9*: EGFP-Bim α2; *10*: EGFP-Bim α3.

FHC-RFP and GFP-Bim L were co-expressed in HEK293 cell. EGFP-Bim L distributed evenly in the cytoplasm, and Ferritin-RFP distributed in the cytoplasm as some particles around the nucleoli. These suggested that FHC and Bim L were spatially accessible (Figure 3).

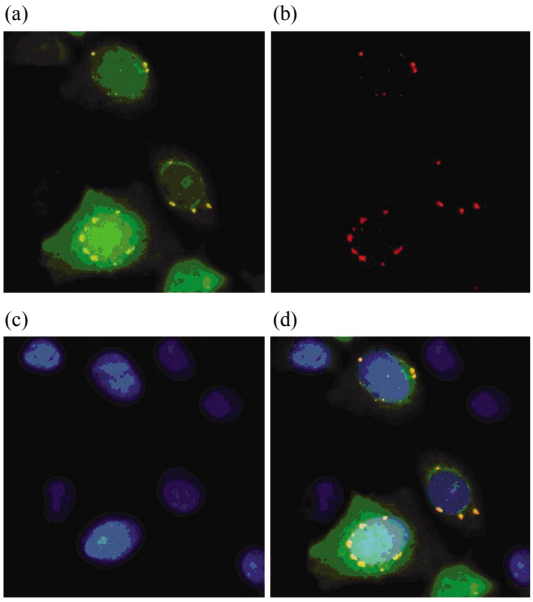

Fig. 3　The subcellular localization of EGFP-Bim L and dsRFP-FHC in HEK293 cells

(a) Subcellular localization of EGFP-Bim L. (b) Subcellular location of DsRed-FHC. (c) Cells co-expressed dsRFP-FHC and EGFP-Bim L stained by DAPI. (d) The merge of (a), (b) and (c).

2.2　Verified the effect of FHC specific siRNA and Bim specific siRNA

Three FHC specific siRNA and three Bim specific siRNA were used to knock down the expression of FHC and Bim. Three siRNA targeting to FHC present down-regulate expression and the FTH1-HSS177645 targeting to fragment (NM_002032; 346-370) encoding the N terminus of FHC was the best, which down regulated 80% FHC expression (Figure 4).

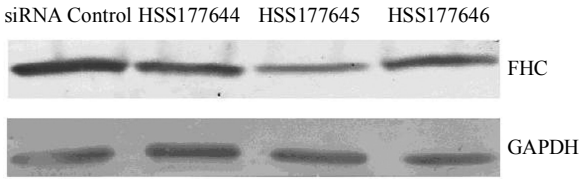

Fig. 4　Verifying the FHC specific siRNA in HEK cells

Three Bim specific siRNA and a scramble siRNA in 6 μmol/L concentration used to knock down the expression of FHC. The blots were detected by a Rabbit anti-FHC antibody.

As to the Bim specific siRNA, two siRNAs (BCL2L11-HSS173328 and BCL2L11-HSS173329) down regulated the GFP-Bim L expression about 70%, while the control scramble siRNA and the BCL2L11-HSS145413 presented no effect on the expression of GFP-Bim L (Figure 5).

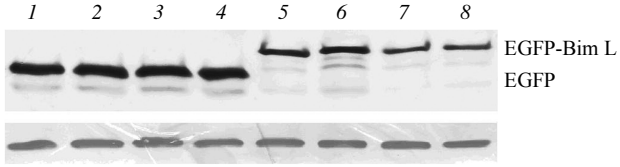

Fig. 5　Verifying the Bim specific siRNA in HEK 293 cells

Three Bim specific siRNA and a scramble siRNA in 6 μmol/L concentration used to knock down the expression of EGFP-Bim L. These siRNA were also used to EGFP expressed cells as control. *1*: siRNA control; *2*: HSS145413; *3*: HSS173328; *4*: HSS173329; *5*: siRNA control; *6*: HSS145413; *7*: HSS173328; *8*: HSS173329.

2.3　FHC protecting cells from apoptosis induced by Bim over expression

HEK293 cells transfected with pEGFP C3, pCMV-Myc-FHC and FHC specific siRNA (FTH1-HSS177645) presented no apoptosis. The HEK293 cells transfected with pEGFP-Bim L/pEGFP-Bim S, pCMV-Myc and control siRNA resulted in more than half cell apoptosis (EGFP-Bim L (60.777 ±0.710)% ; EGFP-Bim S (61.570 ± 2.010)%). Co-transfected HEK293 cells with pCMV-Myc-FHC, pEGFP-Bim L/pEGFP- Bim S and control siRNA protected cells

from the apoptosis caused by over expressing EGFP-BimL/EGFP-Bim S fusion proteins (EGFP-BimL/FHC (50.623 ± 0.710)%, $P < 0.01$; EGFP-BimS/FHC (46.147 ± 1.271)%, $P < 0.01$). In the group transfected with pEGFP-Bim L/pEGFP-Bim S, pCMV- Myc and FHC specific siRNA, more than 70% cells (EGFP-BimL/FTH1-HSS177645(73.157 ± 3.073)%, $P < 0.01$; EGFP-Bim S/FTH1-HSS177645 (74.137 ± 3.181)%, $P < 0.01$) underwent apoptosis (Figure 6).

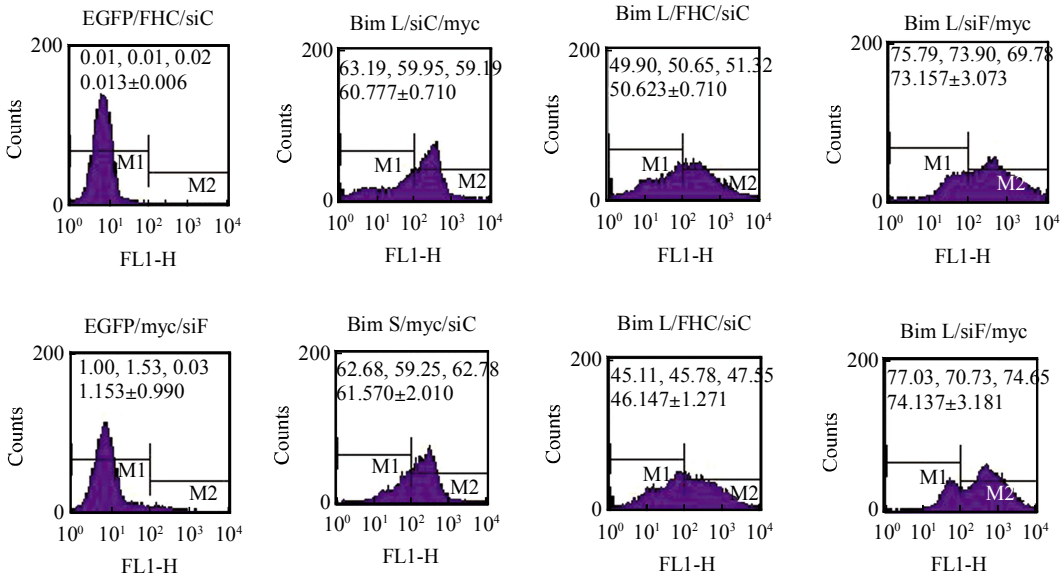

Fig. 6 FHC protects cells from apoptosis induced by overexpressing Bims

siC refer to Control siRNA, siF refer to FHC specific siRNA FTH1-HSS177645, Bim L/S refer to pEGFP-Bim L/S, FHC refer to pCMV-FHC, myc refer to pCMV-Myc.

2.4 FHC and Bim play a role in apoptosis caused by hydrogen peroxide treated

We tested the effect of FHC and Bim on hydrogen peroxide treated HEK293 cells. Knock down the expression of Bims presented no effect on hydrogen peroxide induced apoptosis (BCL2L11-HSS173328 (38.12 ± 3.35)%, control siRNA (38.12 ± 2.45)%, $P > 0.05$, while the cells over-expression of Bim presented more serious apoptosis (76.16 ±1.41)%. In contrast, over-expression of FHC reduced the rate to (26.61 ± 1.98)% ($P < 0.01$). Knock down the expression of FHC resulted in increased apoptosis ((55.28 ± 2.67)%, $P < 0.01$)(Figure 7).

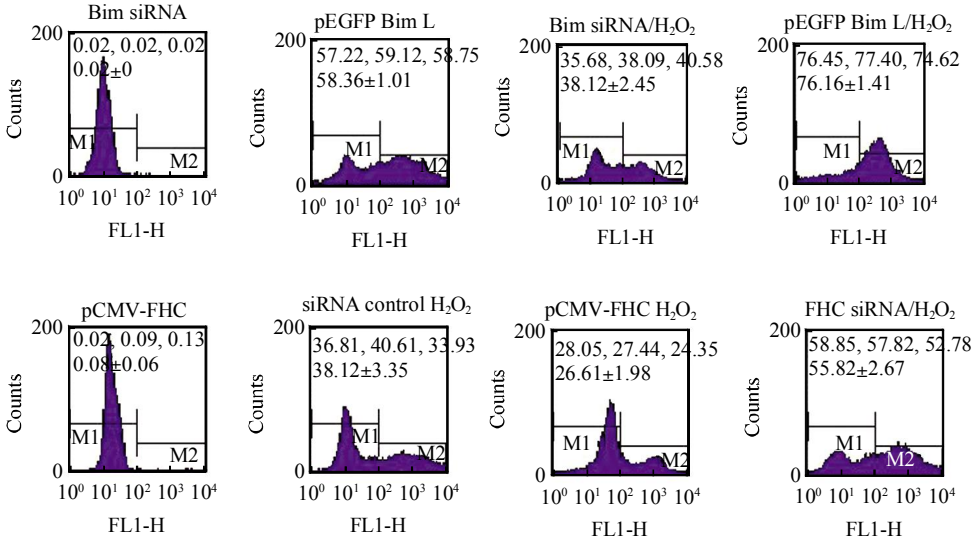

Fig. 7 FHC protects cells from H_2O_2 treatment

Bim siRNA refer to Bim specific siRNA BCL11 HSS173328, FHC siRNA refer to FHC specific siRNA FTH1-HSS177645.

3 Discussion

We isolated FHC as a novel Bim interacting protein by yeast-two-hybridization assay. The interacting fragment on Bim was located to BH3 domain by yeast mating assay. The interaction was verified by co-immunoprecipitation. The subcellular location of Bim L and FHC was partially over-lapped that suggested these two proteins are spatially accessible. The results from both over expression and knock down FHC tests that suggest FHC partially protected cells from apoptosis induced by over expression Bim or ROS.

Ferritins, the major intracellular storage proteins of iron, play a critical role for iron metabolism [21-22]. Altered iron homeostasis is associated with neurodegenerative, retinal degenerative and cardiovascular diseases[23-25]. Deletion of ferritin gene in mice is lethal[23-24]. However, FHC's role in cells should be far more than iron storage cage proteins. It is more likely a part of the surviving mechanism of cells [26-27]. NF-κB protected cells from TNFα also involving tight regulation of FHC expression[28]. Sade[29] reported that ROS regulated quiescent T-cell apoptosis *via* Bim. A recent research suggests that the cyto-protective activity of FHC was closely related to ROS [30]. The question left is that ROS, FHC, Bim involved in apoptosis and where will they converge? It was not surprised us that FHC could protect cells from over-expressing Bims, also, FHC showed protection against apoptosis induced by hydrogen apoptosis. The interaction between Bim and FHC showed us an interesting clue, which might be a good start point to pursue.

Acknowledgements The authors sincerely thank Dr. LI Hong-Bao for her help in preparing the cell cultures.

References

[1] Green D R, Reed J C. Mitochondria and apoptosis. Science, 1998, **281**(5381): 1309-1312

[2] Cory S, Huang D C, Adams J M. The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene, 2003, **22**(53): 8590-8607

[3] O′Connor L, Strasser A, O′Reilly L A, *et al*. Bim: a novel member of the Bcl-2 family that promotes apoptosis. EMBO J, 1998, **17**(2): 384-395

[4] Miyashita T, Shikama Y, Tadokoro K, *et al*. Molecular cloning and characterization of six novel isoforms of human Bim, a member of the proapoptotic Bcl-2 family. FEBS Lett, 2001, **509**(1): 135-141

[5] Chen J Z, Ji C N, Gu S H, *et al*. Over-expression of Bim alpha3, a novel isoform of human Bim, result in cell apoptosis. Int J Biochem Cell Biol, 2004, **36**(8): 1554-1561

[6] Adachi M, Zhao X, Imai K. Nomenclature of dynein light chain-linked BH3-only protein Bim isoforms. Cell Death and Differentiation, 2005, **12**: 192-193

[7] 张 岚, 邢 达. JNK/Bim/Bax 途径在 TNF-α 诱导分化 PC12 细胞凋亡过程中的作用机制. 生物化学与生物物理进展, 2010, **37**(4): 370-380
Zhang L, Xing D. Prog Biochem Biophys, 2010, **37**(4): 370-380

[8] Strasser A, Puthalakath H, Bouillet P, *et al*. The role of bim, a proapoptotic BH3-only member of the Bcl-2 family in cell-death control. Ann N Y Acad Sci, 2000, **917**: 541-548

[9] Wang P, Gilmore A P, Streuli C H. Bim is an apoptosis sensor that responds to loss of survival signals delivered by epidermal growth factor but not those provided by integrins. J Biol Chem, 2004, **279**(40): 41280-41285

[10] Czabotar P E, Colman P M, Huang D C. Bax activation by Bim?. Cell Death Differ, 2009, **16**(9): 1187-1191

[11] Nagaprashantha L D, Vatsyayan R, Lelsani P C, *et al*. The sensors and regulators of cell-matrix surveillance in anoikis resistance of tumors. Int J Cancer, 2011, **128**(4): 743-752

[12] Bouillet P, Huang D C, O′Reilly L A, *et al*. The role of the pro-apoptotic Bcl-2 family member bim in physiological cell death. Ann N Y Acad Sci, 2000, **926**: 83-89

[13] Matsui H, Asou H, Inaba T. Cytokines direct the regulation of Bim mRNA stability by heat-shock cognate protein 70. Mol Cell, 2007, **25**(1): 99-112

[14] Hu Z Q, Zhang J Y, Ji C N, *et al*. Grb10 interacts with Bim L and inhibits apoptosis. Mol Biol Rep, 2010, **37**(7): 3547-3552

[15] Okuno S, Saito A, Hayashi T, *et al*. The c-Jun N-terminal protein kinase signaling pathway mediates Bax activation and subsequent neuronal apoptosis through interaction with Bim after transient focal cerebral ischemia. J Neurosci, 2004, **24**(36): 7879-7887

[16] Mérino D, Giam M, Hughes P D, *et al*. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. J Cell Biol, 2009, **186**(3): 355-362

[17] Lei K, Davis R J. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. Proc Natl Acad Sci USA, 2003, **100**(5): 2432-2437

[18] Pham C G, Bubici C, Zazzeroni F, *et al*. Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. Cell, 2004, **119**(4): 529-542

[19] Aykin-Burns N, Ahmad I M, Zhu Y, *et al*. Increased levels of superoxide and H_2O_2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. Biochem J, 2009, **418**(1): 29-37

[20] Richardson D R. 24p3 and its receptor: dawn of a new iron age?. Cell, 2005, **123**(7): 1175-1177

[21] Nakano H. A revival of old players. EMBO Rep, 2005, **6**(2): 126-

127

[22] Fisher J, Devraj K, Ingram J, *et al*. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. Am J Physiol Cell Physiol, 2007, **293**(2): C641−C649

[23] Ferreira C, Bucchini D, Martin M E, *et al*. Early embryonic lethality of H ferritin gene deletion in mice. J Biol Chem, 2000, **275**(5): 3021−3024

[24] Ferreira C, Santambrogio P, Martin M E, *et al*. H ferritin knockout mice: a model of hyperferritinemia in the absence of iron overload. Blood, 2001, **98**(3): 525−532

[25] Papa S, Bubici C, Pham C G, *et al*. NF-kappaB meets ROS: an 'iron-ic' encounter. Cell Death Differ, 2005, **12**(10): 1259−1262

[26] Shackelford R E, Manuszak R P, Johnson C D, *et al*. Iron chelators increase the resistance of Ataxia telangeictasia cells to oxidative stress. DNA Repair (Amst), 2004, **3**(10): 1263−1272

[27] Chen M, Awe O O, Chen-Roetling J, *et al*. Iron regulatory protein-2 knockout increases perihematomal ferritin expression and cell viability after intracerebral hemorrhage. Brain Res, 2010, **1337**: 95−103

[28] Ventura J J, Cogswell P, Flavell R A, *et al*. JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. Genes Dev, 2004, **18**: 2905−2915

[29] Sade H, Sarin A. Reactive oxygen species regulate quiescent T-cell apoptosis via the BH3-only proapoptotic protein BIM. Cell Death Differ, 2004, **11**(4): 416−423

[30] Arosio P, Levi S. Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. Biochim Biophys Acta, 2010, **1800**(8): 783−792

FHC 和 Bim 相互作用抵抗细胞凋亡 *

邹冠玉 季朝能 沈 琪 薛京伦 陈金中 **

(复旦大学生命科学学院遗传工程国家重点实验室，上海 200433)

摘要 FHC 和 Bim 参与细胞铁代谢和由 ROS 引起的细胞凋亡过程．但是其具体的分子机制还未阐明．用 pLexA-Bim L 作为诱饵，筛选了一个基于 pBD42AD 的胎脑 cDNA 文库，发现 FHC 是一个新的 Bim 相互作用蛋白．酵母杂交实验发现 Bim 的相互作用片段为 BH3 功能域．上述相互作用进一步用免疫共沉淀和荧光共定位得以证实．在 HEK293 细胞过表达 FHC 可以减轻由 Bim 过表达或 ROS 所引起的细胞凋亡，而用 FHC 特异性 siRNA 调低 FHC 表达，则增加 Bim 过表达或 ROS 引起的细胞凋亡．研究首次报道了 Bim 和 FHC 的相互作用以及对细胞凋亡和氧化应激的影响，为进一步阐明 FHC 和 Bim 参与凋亡和 ROS 反应提供了新的线索．

关键词 铁蛋白重链 FHC，Bim，氧化应激，细胞凋亡

学科分类号 G633.91，Q2，Q7 **DOI**: 10.3724/SP.J.1206.2010.00608

* 国家高技术研究发展计划(863)(2009ZX09503-20)和国家自然科学基金(30971617)资助项目.

** 通讯联系人.

Tel: 021-65642424, E-mail: kingbellchen@fudan.edu.cn

收稿日期：2011-02-28，接受日期：2011-04-12