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Different gene expression profiles of AD293 and HEK293 cell lines that show contrasting susceptibility to apoptosis induced by overexpression of Bim L

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Bim is a pro-apoptotic member of the Bcl-2 protein family. Overexpression of Bim proved to be highly cytotoxic for diverse cells. The AD293 cell line is derived directly from the HEK293 cell line but has been transfected with a gene that can improve cell adherence. We found that there was almost no apoptosis seen in Bim L-transfected AD293 cells, but more than half of Bim L-transfected HEK293 cells underwent apoptosis. Suppression subtractive hybridization was used to detect the different gene expression profile between these two cell lines. In 192 sequenced positive clones, there were 30 clones repeating twice or more. Ten genes were selected for identification by semi-quantitative RT-PCR. The transcripts of two adhesion-related genes (actin and parvin) and two apoptosis-related genes (cyclin 2 and protein phosphatase 1G) were up-regulated in AD293 cells. These results suggest that the high expression of cell adhesion-related proteins might be responsible for the different apoptosis status after the transfection of Bim L. Our data provide candidate genes responsible for the different apoptosis sensitivity of these two cell lines. Further investigation on the differential expression profile between AD293 and HEK293 might improve our understanding of cell apoptosis mechanism.

Keywords: expression profile, SSH, Bim L, AD293, apoptosis

INTRODUCTION

Apoptosis, the physiological process of cell death, is critical for modeling tissues and maintaining homeostasis in multicellular organisms (Kerr *et al*., 1972). Deregulation of apoptosis may cause diseases, such as cancers, immune diseases, and neurodegenerative disorders. Signaling for apoptosis occurs through multiple independent pathways that are initiated either from triggering events within the cell or from outside the cell. All apoptosis signaling pathways converge on a common mechanism of cell destruction that is activated by a family of cysteine proteases (caspases) that cleave proteins at aspartate residues (Strasser *et al*., 2000). Although the caspases represent a central point in apoptosis, their activation is regulated by a variety of other factors. Among these, Bcl-2 family plays a pivotal role in caspases' activation, by this deciding whether a cell will live or die (Burlacu, 2003). The Bcl-2 protein family contains pro- and anti-apoptotic proteins that regulate the mitochondrial pathway of apoptosis (Antonsson, 2004; Vaux *et al*., 1988; Adams & Cory, 1998). The balance between these counteracting proteins presumably determines the cell fate (Oltvai *et al*., 1993; Thompson, 1995; White, 1996).

Bim is a 'BH3-only' pro-apoptotic protein of Bcl-2 family in the mitochondria pathway (O'Connor *et al*., 1998). To date, eleven isoforms of Bim have been reported (Mami *et al*., 2001; JinZhong *et al*., 2004). Three classical isoforms (Bim EL, Bim L and Bim S) contain a hydrophobic C-terminal region and

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Abbreviation: GAPD, glyceraldehyde-3-phosphate dehydrogenase; G3PDH, glycerol-3-phosphate dehydrogenase; PMSA, prostate-specific membrane antigen; SSH, suppresssion substrate hybridization.

a BH3 domain (O'Connor *et al*., 1998; Puthalakath *et al*., 1999). Resent research showed that Bim could induce apoptosis through direct interaction with Bax as well as through interaction with Bcl-2 and Bcl-X_L (O'Connor *et al*., 1998; O'Reilly *et al*., 2000; Marani *et al*., 2002). To investigate the role of Bim in cell apoptosis, we transiently transfected HEK293 and AD293 cells with the plasmid pcDNA3.0-Bim L. Overexpression of Bim L can induce apoptosis in about half of HEK293 cells but cannot induce AD293 cells' apoptosis. The AD293 cell line is derived directly from the HEK293 cell line by transfection with a gene that improved cell adherence (Graham *et al*., 1977). The different apoptosis susceptibility of these two cell lines to Bim L promotes us to pursue further study. Here we report a differential gene expression profile obtained by a suppression subtractive hybridization between HEK293 and AD293 cells.

MATERIALS AND METHODS

Cell culture, transfection, Western blot analysis and apoptosis assays. HEK293 and AD293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 50 μg streptomycin/ml, and 50 IU penicillin/ml. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2.

HEK293 and AD293 cells were plated at a density of 2×10^5 cells per well. When the plates were about 75% confluent, cells were transfected with pcDNA3.0-Bim L or pcDNA3.0 using the Lipofectamine agent (Invitrogene). Twenty-four hours after transfection, the cells were harvested. Apoptotic cells were quantified by flow cytometric analysis (APO-BRDU^{TM} Apoptosis Assay Kit, Becton Dickinson) according to the manufacturer's recommendations. Total cell extracts were resolved by SDS/PAGE. The expression of Bim L was detected by immunoblotting with a human Bim EL specific rabbit polyclonal antibody (Santa Cruz Biotechnology) followed by horseradish peroxidase-conjugated anti-rabbit antibody (Sigma-Aldrich). The proteins were visualized by using enhanced chemiluminescence immunoblotting detection reagents (Amersham). The blots were also developed with an anti-actin rabbit polyclonal antibody (Sigma-Aldrich) as a control.

Suppression subtractive hybridization between HEK293 and AD293 cells. AD293 and HEK293 cells were cultured in five 25 ml culture flasks. Cells were seeded at a density of 2×10^6/ml. Total RNA was extracted by using Trizol agent (GIBCOL). mRNA was isolated by using Oligotex mRNA Kits (QIAGEN). The PCR cDNA subtraction kit and Advantage 2 DNA polymerase were purchased from Clontech. Both the mRNA of AD293 and HEK293 were used as driver and tester for constructing forward and reverse libraries. Muscle mRNA and *Hae* III-digested Φx 174 DNA testers and muscle mRNA driver were employed as the control panel. The SSH were performed with a standard procedure recommended by the manufacturer. Each of 192 independent clones of AD293 forward and reverse libraries was amplified with M13 consensus primers and immobilized on an Immobilon-Ny+ membrane (Millipore) with 50 ng/clone per spot. The blots were detected with probes of the PCR products amplified with M13 consensus primers both from forward and reverse libraries. The probes were labeled with (α-^{32}P)-dATP using a random primer DNA labeling kit (Worthington). The clone identity was verified by sequencing the corresponding plasmids with M13 consensus primers. All draft sequences were identified by Blast analysis against NCBI database (www.ncbi.nlm.nih.gov).

RT-PCR. Transcripts of 10 different expressed genes between AD293 and

Table 1. The primer sequences for RT-PCR.

Gene identity	Primer sequence	Expected amplified fragment length
NM_014739	5′-gttgtgtgtgtgttaaaaggagc-3′ 5′-atcaccctagcctactacactctg-3′	666 bp
NM_018222	5′-ttgtagccatcaactgactgagacc-3′ 5′-ctgggctgagacaaagaactcaatc-3′	809 bp
NM_018947	5′-agtggctagtggctactgtattgac-3′ 5′-cacgacgcccagttatctagtttac-3′	757 bp
NM_001909	5′-gaccagaacatcttctccttctacc-3′ 5′-ggtcttccaatgcacgaaacagatc-3′	840 bp
NM_001614	5′-ccggaattcatggaagaagagatcgccgcgctg-3′ 5′-cgcggatccgaagcatttgcggtggacgatggag-3′	1124 bp
NM_002788	5′-ccggaattcatgagctcaatcggcactgggtatg-3′ 5′-cgcggatcccatattatcatcatctgattcatcttcttc-3′	764 bp
NM_001166	5′-ggacctggagaaagttcttcagaag-3′ 5′-agagagaaatgtacgaacagtaccc-3′	722 bp
NM_002082	5′-tcactttgctcacagctcctctgc-3′ 5′-atgctctggtcgctatatacattcg-3′	765 bp
NM_002707	5′-aagaagaagagatgatggtgccagg-3′ 5′-tggatgactgctagtctcgcttgg-3′	728 bp
XM_043885	5′-agatttactactaggaaaccttcctc-3′ 5′-ctcctgcacttagcaaaaatctgtc-3′	544 bp
G3PDH	5′-tgaaggtcggagtcaacggatttggt -3′ 5′-catgtgggccatgaggtccaccac-3′	983 bp

HEK293 cells were selected out to identify by semi-quantitative RT-PCR. The first-strand cDNAs were synthesized using AMV Reverse Transcriptase (Promega). Then, 10 pairs of gene specific primers (Table 1) and Advantage 2 DNA polymerase (Clontech) were used to amplify the cDNAs, and G3PDH primers (Table 1) were used to amplify the same cDNAs as a control. The amplification conditions were as follows: 94°C 30 s, 65°C 30 s, 72°C 90 s, 30 cycles. The relative quantities of the specific PCR products were determined by densitometric analysis and normalized with the density of the G3PDH product. The targeted PCR products were cloned into T-vector and sequenced with both M13 consensus primers.

RESULTS

Different apoptotic characters of AD293 and HEK293 cells

AD293 and HEK293 cells were transfected with pcDNA3.0-Bim L. Western blot analysis showed that both AD293 and HEK293 cells overexpressed Bim L protein, moreover, the expression of Bim L in AD293 has higher (Fig. 1A). Overexpression of Bim L did not induce AD293 apoptosis (0.667 ± 0.601%) cells. The control empty pcDNA3.0 plasmid caused apoptosis of 0.237 ± 0.076%. In contrast, overexpression of Bim L in HEK293 caused significantly apoptosis (56.887 ± 0.287%). The control pcDNA3.0 plasmid caused apoptosis in only 0.427 ± 0.045% cells (Fig. 1B).

Differential gene expression profile between AD293 and HEK293 cells

Using the PCR products from AD293 cells forward subtraction library as probes, we detected the forward and reverse subtraction library, respectively, and found that genes in these two libraries were significantly different (Fig. 2). The result indicated that the SSH we performed was credible. In 192 sequenced clones, there were 30 clones repeating twice or more. Among these clones, there were two cell adhesion-related genes, ten cell apoptosis-related genes, two cell cycle-related genes and three neurodegenerative diseases-related genes (Table 2).

By performing semi-quantitative RT-PCR analysis, we found that among 10 up-regulated genes came from the AD293 forward library, the transcripts of 7 genes were clearly up-regulated. One of the ten had shared a similar expression level in both cell lines. In lane 3, there was a weak band in AD293 sample but no band in HEK293 sample.

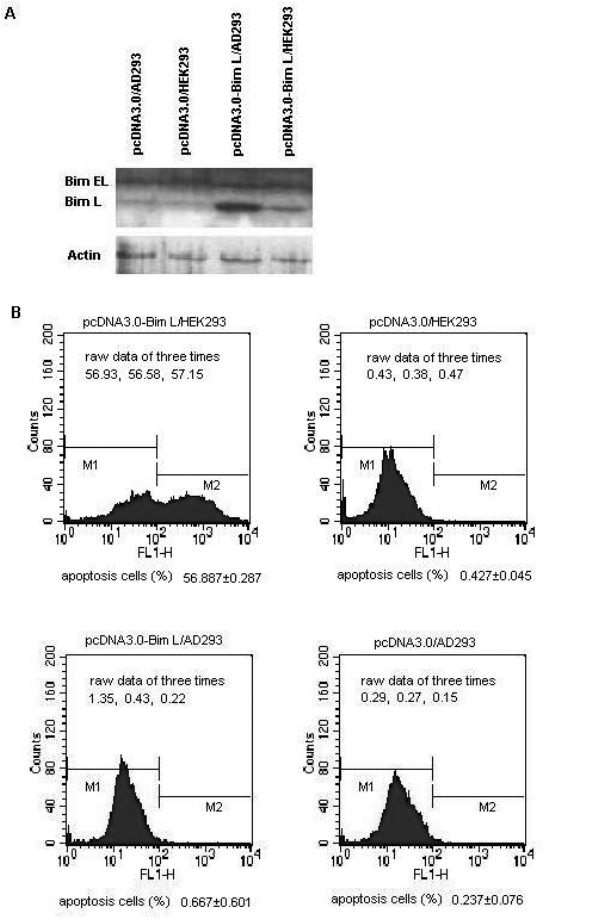

Figure 1. Different responses of AD293 and HEK293 to Bim L-mediated apoptosis.
A. Western blot analysis of AD293 and HEK293 cells transfected with Bim L. The expression of Bim L was detected by immunoblotting with an anti-Bim polyclonal antibody. A parallel blot for actin served as loading control. B. Apoptosis assay of HEK293 and AD293 cells transfected with pcDNA3.0-Bim L or pcDNA3.0. Each group repeated three times.

All bands were verified by sequence analysis. An unexpected band of about 2 kb appeared in lane 8 of the AD293 sample. This turned out to be an intron containing fragment corresponding to a region of human cytoskeletal gamma actin gene, (ACTG1, M19283). The transcripts of the G3PDH control were almost of the same intensity in the two cell lines (Fig. 3).

DISCUSSION

HEK293 cells are human embryonic kidney cells transformed by sheared adenovirus type 5 DNA (Graham *et al*., 1977). The HEK293 cell line and its sublines are widely used in molecular and cellular research. AD293 cells constructed by the Stratagene Company for adenovirus transfection system are de-

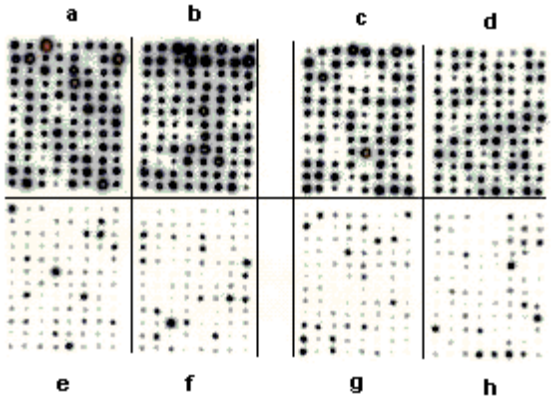

Figure 2. Dot blot of the SSH between AD293 and HEK293 cells.
a, b) Using the PCR products from AD293 forward library as probes, AD293 forward clone blots were detected; c, d) using the PCR products from AD293 reverse library as probes, HEK293 forward clone blots were detected; e, f) AD293 forward clone blots were detected by probes from AD293 reverse library; g, h) HEK293 forward clone blots were detected by probes form AD293 forward library.

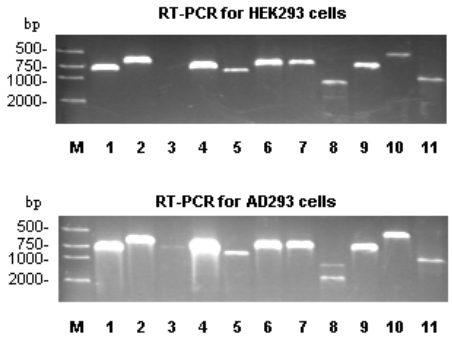

Figure 3. RT-PCR of 10 selected genes expressed in AD293 forward library.
Lane 1. NM_018222; 2. NM_014739; 3. NM_002082; 4. NM_002788; 5. NM_001909; 6. NM_002707; 7. NM_001166; 8. NM_001614; 9. NM_018947; 10. XM_043885; 11. G3PDH; M. DNA marker.

rived from the commonly used HEK293 cell line but by transfection with a gene that can improve cell adherence and plaque formation properties (Adeasy XL adenoviral vector system, catalog 240010).

We transfected AD293, HEK293, glioma cell line U251, human liver cancer cell line 7721 and rat hepatocellular carcinoma cell line 7919 with pCDNA-Bim L. As expected, we observed that Bim L induced HEK293 (Fig. 1B), U251, 7721 and 7919 (not show) cells apoptosis significantly which was consistent

Table 2. Analysis of up-regulated gene library in AD293 cells

Gene	Gene background	Frequency
NM_001695	ATPase, H+ transporting, lysosomal	6
XM_044421	solute carrier family 19 (thiamine transporter)	5
NM_003576	serine/threonine kinase 24 (STE20 homolog, yeast)	4
NM_000979	ribosomal protein L18	3
NM_001226	caspase 6	3
NM_004522	kinesin family member 5C	3
NM_006827	transmembrane trafficking protein	3
NM_018222	parvin, alpha (PARVA), (adherin)	3
NM_024050	hypothetical protein MGC2594	3
NM_002788	PMSA3	2
XM_043885	DKFZP564F0522 protein	3
BC016333	cyclin L2	2
HSMB1	lymphocyte lineage-restricted mb-1 membrane glycoprotein C-term (CD3 like)	2
NM_001166	baculoviral IAP repeat-containing 2 (BIRC2)	2
NM_001614	actin, gamma 1	2
NM_001909	cathepsin D (lysosomal aspartyl protease)	2
NM_002046	GAPD	2
NM_002082	G protein-coupled receptor kinase 6	2
NM_002707	protein phosphatase 1G (formerly 2C), magnesium-dependent	2
NM_004238	thyroid hormone receptor interactor 12	2
NM_004893	H2A histone family, member Y	2
NM_014739	Bcl-2-associated transcription factor (BTF)	2
NM_014762	24-dehydrocholesterol reductase (DHCR24)	2
NM_015070	KIAA0853	2
NM_018947	cytochrome *c*, somatic	2
XM_051720	BTAF1 RNA polymerase II	2
XM_084349	LOC196692	2
XM_098401	LOC153606	2
XM_106318	LOC166889	2
XM_115874	similar to cytochrome oxidase I (LOC203438)	2

with Mami's and Chen's results (Mami *et al*., 2001; JinZhong Chen *et al*., 2004). However, overexpression of Bim L in AD293 cells, a cell line derived directly from HEK293, did not induce apoptosis (Fig. 1B). Compared with HEK293 cells transfected with the empty pcDNA3.0 plasmid, AD293 cells transfected with the same empty plasmid showed a lower apoptosis frequency. Moreover, AD293 cells also resist antibiotic G418 selection and serum deprivation (not show). Compared with transfected HEk293 cells, transfected AD293 cells expressed Bim L protein at an even higher lever (Fig. 1A). This prompted us to explore the differences between the AD293 and HEK293 cell line.

We performed a SSH to detect the gene expression difference between these two cell lines. Using PCR products from the AD293 forward subtraction library as probes, a dot blot was performed. In 192 positive sequenced clones, there were 30 clones repeating twice or more (Table 2). Among these clones, there were seven putative genes, six housekeeping genes, two cell cycle-related genes (cyclin L2 and FIN), three neurodegenerative diseases-related genes (kinesin family member 5C, transmembrane trafficking protein and DHCR24), two cell adhesion-related genes (actin and parvin), ten apoptosis related-genes (LOC203438, cytochrome *c*, caspase 6, BIRC2, STE20 homolog, BTF, PMSA3, ATPase, cathepsin D and G protein-coupled receptor kinase 6).

We performed an RT-PCR analysis of 10 genes involved to determine the reliability of the library produced by SSH. Both the dot blot and RT-PCR results suggested that the SSH library was reliable (Figs. 2, 3). All ten PCR products were verified by sequencing. As to the unexpected band of about 2 kb appearing in lane 8 of the AD293 sample, we speculated that this unexpected sequence in AD293 came from a plasmid containing ACTG1 (actin, gamma 1) which is tranfected into AD293 cells, an hnRNA or genomic DNA contamination (Erba *et al*., 1988). The RNA extraction of AD293 and RT-PCR of ACTG1 were preformed three times and the results showed no significant difference (not shown).

The loss of cell adherence results probably from the rearrangement of the cytoskeleton caused by proteolysis of actin or fodrin (Monteiro *et al*., 2004) and the loss of adherence ability in adherent cells is one reason for apoptosis initiation (Puthalakath *et al*., 2001). There were two cell adhesion-related genes (actin, parvin) up-regulated in AD293 cells. The high expression of these cell adhesion-related proteins might be responsible for the different apoptosis status after the transfection of Bim L. Our research was consistent with that of Collins and Fukazawa (Fukazawa *et al*., 2004; Collins *et al*., 2005).

There were five apoptosis-inhibiting genes up-regulated in AD293 cells. BTF encoded by NM_014739 can inhibit the transcription of bcl-2 apoptotic genes (Kasof *et al*., 1999). PSMA3 encoded by NM_002788 can prevent cell apoptosis through accelerating the degradation of apoptotic proteins (Wakako *et al*., 2001). NM_001695 and NM_001909 encode ATP6V1C1 and cathepsin D, respectively. The former is associated with the H^+ regulation of lysosome and is supposed to prevent apoptosis by inhibiting cytoplasm acidification (Izumi *et al*., 2003). The latter triggers Bax activation and Bax induces the selective release of mitochondrial AIF which is responsible for the early apoptotic phenotype (Bidere *et al*., 2003). cIAP encoded by NM_001166 may be a factor in the presumed anti-apoptotic system in human gastric cancer cells (Endo *et al*., 2005) and has been suggested to play important roles in the development of cervical and esophageal squamous cell carcinomas (Tanimoto *et al*., 2005).

AD293, HEK293 transfected with an adherence gene, presents higher expression of adherence-related proteins and anti-apoptosis proteins. It resisted apoptosis signals such as over-expression of Bim L and growth factors deprivation. Our results suggest that the anti-apoptotic activity of enhanced cell adherence plays a role both in neutralizing pro-apoptotic molecules and in up-regulating expression of anti-apoptotic proteins. Further investigation might improve our understanding of cell apoptosis mechanism.

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REFERENCES

Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. *Science* **281**: 1322–1326.

Antonsson B (2004) Mitochondria and the Bcl-2 family proteins in apoptosis signaling pathways. *Mol Cell Biochem* **256–257**: 141–155.

Bidere N, Lorenzo HK, Carmona S, Laforge M, Harper F, Dumont C, Senik A (2003) Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. *J Biol Chem* **278**: 31401–31411.

Burlacu A (2003) Regulation of apoptosis by Bcl-2 family proteins. *J Cell Mol Med* **7**: 249–257.

Collins NL, Reginato MJ, Paulus JK, Sgroi DC, Labaer J, Brugge JS (2005) G1/S cell cycle arrest provides anoikis resistance through Erk-mediated Bim suppression. *Mol Cell Biol* **25**: 5282–5291.

Endo K, Kohnoe S, Tsujita E, Watanabe A, Nakashima H, Baba H, Maehara Y (2005) Modulation of anti-apopto-

sis by endogenous IAP expression in MKN45 human gastric cancer cells. *Anticancer Res* **25**: 2713–2717.

Erba HP, Eddy R, Shows T, Kedes L, Gunning P (1988) Structure, chromosome location, and expression of the human gamma-actin gene: differential evolution, location, and expression of the cytoskeletal beta- and gamma-actin genes. *Mol Cell Biol* **8**: 1775–1789.

Fukazawa H, Noguchi K, Masumi A, Murakami Y, Uehara Y (2004) BimEL is an important determinant for induction of anoikis sensitivity by mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitors. *Mol Cancer Ther* **3**: 1281–1288.

Graham FL, Smiley J, Russell WC, Nairn R (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Viral* **36**: 59–74.

Izumi H, Ise T, Murakami T, Torigoe T, Ishiguchi H, Uramoto H, Yoshida Y, Yoshida T, Tanabe M, Kohno K (2003) Structural and functional characterization of two human V-ATPase subunit gene promoters. *Biochim Biophys Acta* **628**: 97–104.

JinZhong Chen, ChaoNeng Ji, ShaoHua Gu, JiXi Li, En-Peng Zhao, Huang Y, Huang L, Ying K, Xie Y, Mao YM (2004) Over-expression of Bim_3, a novel isoform of human Bim, result in cell apoptosis. *Int J Biochem Cell Biol* **36**: 1554–1561.

Kasof GM, Goyal L, White E (1999) Btf, a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins. *Mol Cell Biol* **19**: 4390–4404.

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis, a basic biological phenomenon with wide-ranging implications in tissue kinetics *Br J Cancer* **26**: 239–257.

Mami U, Miyashita T, Shikama Y, Tadokoro K, Yamada M (2001) Molecular cloning and characterization of six novel isoforms of human Bim, a member of the pro-apoptotic Bcl-2 family. *FEBS Lett* **509**: 135–141.

Marani M, Tenev T, Hancock D, Downward J, Lemoine NR (2002) Identification of novel isoforms of the BH3 domain protein Bim which directly activate Bax to trigger apoptosis. *Mol Cell Biol* **22**: 3577–3589.

Monteiro HP, Silva EF, Stern A (2004) Nitric oxide: apotential inducer of adhesion-related apoptosis-anoikis. *Nitric Oxide* **10**: 1–10.

O'Connor L, Strusser A, O'Reilly LA, Hausman J, Adams JM, Cory S, Huang DC (1998) Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J* **17**: 384–395.

Oltvai ZN, Milliman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74**: 609–619.

O'Reilly LA, Cullen L, Visvader J, Lindeman GJ, Print C (2000) The proapoptotic BH3-only protein bim is expressed in hematopoietic, epithelial, neuronal, and germ cells. *J Pathol* **157**: 449–461.

Puthalakath H, Huang D, O'Reilly LA, King SM, Strasser A (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol Cell* **3**: 287–296.

Puthalakath H, Villunger A, O'Reilly LA, Beaumont JG, Coultas L, Cheney RE, Huang DC, Strasser A (2001) Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science* **293**: 1829–1832.

Strasser A, O'Connor L, Dixit VM (2000) Apoptosis signaling. *Annu Rev Biochem* **69**: 217–245.

Tanimoto T, Tsuda H, Imazeki N, Ohno Y, Imoto I, Inazawa J, Matsubara O (2005) Nuclear expression of cIAP-1, an apoptosis inhibiting protein, predicts lymph node metastasis and poor patient prognosis in head and neck squamous cell carcinomas. *Cancer Lett* **224**: 141–151.

Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* **267**: 1456–1462.

Vaux DL, Cory S, Adams JM (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**: 440–442.

Wakako T, Tatsuhiko K, Takao H, Keiji T, Toshiaki S, Hiroyuki A, Naeko M, Noriko N (2001) Anti-atherogenic antioxidants regulate the expression and function of proteasome α-type subunits in human endothelial cells. *J Biol Chem* **276**: 40497–40501.

White E (1996) Life, death, and the pursuit of apoptosis. *Gene* **10**: 1–15.