Functional screening of genes suppressing TRAIL-induced apoptosis: distinct inhibitory activities of Bcl-X<sub>L</sub> and Bcl-2

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Tumour cells express several proteins that suppress apoptosis and thereby become resistant to various forms of therapy. Gene products controlling the balance between cell death and survival arise from an expanding family of genes, of which Bcl-2 family is clearly associated with apoptosis inhibition (Chao and Korsmeyer, 1998). The antiapoptotic members of Bcl-2 gene family exert their antiapoptotic functions by preventing the release of cytochrome c from mitochondria to the cytosol and prevent the loss of mitochondrial outer membrane integrity by blocking both membrane hyperpolarisation and mitochondrial swelling (Vander-Heiden et al., 1997; Harris and Thompson, 2000).

Agents that induce apoptosis in cancer cells have recently attracted great attention. The apoptotic process can be triggered by pleiotropic ways, including activation of tumour necrosis factor receptor (TNF-R) family, γ-irradiation, and various chemotherapeutic agents, etc. The known signalling pathways induced by various apoptotic stimuli converge into a common death pathway either at mitochondrial step or finally at a step at which caspases are activated (Green and Reed, 1998; Thornberry and Lazebnik, 1998). TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family that is capable of inducing apoptosis in tumour cells examined (Wiley et al., 1995; Pitti et al., 1996). In animal model, TRAIL efficiently suppressed tumours with no detectable toxicity, suggesting that it could potentially serve as an useful chemotherapeutic agent (Ashkenazi et al., 1999; Walczak et al., 1999). While some studies raised questions of whether normal cell types were truly protected from TRAIL (Jo et al., 2000; Leverkus et al., 2000), the TRAIL currently being developed for clinical trials does not evoke these cytotoxic effects on hepatocytes (Lawrence et al., 2001; Qin et al., 2001).

TNF-related apoptosis-inducing ligand is highly homologous to FasL and TNF ligand family. Unlike other members, TRAIL is constitutively expressed in most tissues and cells (Wiley et al., 1995). TNF-related apoptosis-inducing ligand-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), and TRAIL-R4 (DcR2) have been identified as TRAIL receptors (Pan et al., 1997a,b; Sheridan et al., 1997; Wu et al., 1997; Mongkolsapaya et al., 1998). While TRAIL-R1 and TRAIL-R2 contain a cytoplasmic death domain, TRAIL-R3 and TRAIL-R4 lack the death domain and bind to TRAIL without activation of apoptotic machinery. Though caspase-8 was recently reported to play a critical role in TRAIL-mediated apoptosis (Kim et al., 2000), the sequence of events occurring downstream of the receptors is not well understood. In the present study, we isolated Bcl-X<sub>L</sub> as an inhibitory gene of TRAIL-induced apoptosis from cDNA library by expression screening assay. We found differential expression pattern of Bcl-2 and Bcl-X<sub>L</sub> in the human breast cancer tissues and present evidences for the inhibitory effects of Bcl-X<sub>L</sub>, but not Bcl-2, on the TRAIL-induced apoptosis of tumour cell lines.

Keywords: TRAIL; apoptosis; functional screening; Bcl-X<sub>L</sub>; caspase
MATERIALS AND METHODS

Cell line and DNA transfection

MDA-MB-231, SK-BR-3, Jurkat, and Jurkat32H cells were grown in RPMI 1640 containing 10% fetal bovine serum (FBS). HeLa cells were incubated with Dulbecco’s modified Eagle’s Medium with 10% FBS. HeLa cells and Jurkat cells permanently expressing either Bcl-2 (HeLa/Bcl-2) or Bcl-X<sub>L</sub> (HeLa/Bcl-X<sub>L</sub>) were established by transfection of expression plasmids of human Bcl-2 and Bcl-X<sub>L</sub>. Cells were transfected with Lipofect-AMINE PLUS™ reagent according to the recommended methods by the manufacturer (Gibco BRL, Grand Island, NY, USA) or with standard CaCl<sub>2</sub> methods. After 1 day, cells were grown in the presence of 700 µg ml<sup>−1</sup> G418 (Gibco BRL) or 1 µg ml<sup>−1</sup> puromycin (Sigma, St Louis, MO, USA) for 3 weeks. Each clone was examined for the expression of exogenous gene with Western blot analysis.

Materials

Rhodamine 123, a cell-permeable mitotracker, was from Molecular Probe Inc. (Eugene, OR, USA). The fluorogenic caspase substrates DEVD-aminomethylcoumarine (AMC), IETD-AMC, and LEHD-AMC were from Enzyme System Products (Livermore, CA, USA). Anticaspase-3 (SC-7148) and anti-Bcl-2 antibody was from DAKO (Copenhagen, Denmark); anti-caspase-8 and -9 antibodies were previously described (Kim et al, 2000); anti-z-tubulin antibody was from Sigma; anti-rabbit IgG-horseradish peroxidase (HRP), anti-mouse IgG-HRP, and anti-goat IgG-HRP antibodies were from Santa Cruz. All other molecular biology grade reagents were from Sigma or New England Biolabs (Hertfordshire, England).

Collection of surgical samples

Tissues were obtained from seven patients who were operated upon infiltrating ductal carcinoma of breast at Department of Surgery, Seoul National University Hospital, Seoul, Korea. Fresh specimens of cancer core tissues and adjacent normal breast tissues from the same patient were immediately frozen in liquid nitrogen and stored at −80°C for Western blot analysis. For the immunohistochemical analysis, samples were formalin-fixed and paraffin-embedded. Parallel samples were processed for histologic examination.

TNF-related apoptosis-inducing ligand preparation

TNF-related apoptosis-inducing ligand preparation was previously described by Cha et al (1999). Briefly, truncated human TRAIL (amino acid 114–281) in pET-3a plasmid was expressed in BL21 (DE3) by 1 mM isopropyl-beta-D-thiogalactoside. After sonication, TRAIL were isolated as insoluble aggregates by centrifugation and then with a biotinylated link antibody (DAKO) for 30 min followed by overnight dialysis in the same buffer at 4°C. After removing aggregates, the supernatant solution was loaded on a SP Sepharose Fast Flow column (Amersham-Pharmacia, Piscataway, NJ, USA). Fractions eluted at 0.8–1.0 M NaCl gradient contained TRAIL almost exclusively as judged by SDS–PAGE.

Cell-based functional screening to isolate inhibitors of TRAIL-induced apoptosis

(a) Retroviral infection of cDNA library and selection for TRAIL-resistant clones A retroviral library containing 2 × 10<sup>6</sup> independent cDNA inserts was constructed from RNA of human lymph node, thymus, spleen, and bone marrow by standard methods, using a retrovirus vector pTRA. Amphotrophic retroviral packaging cells, φ NX-ampho, were transduced with 10 µg of cDNA library using CaCl<sub>2</sub> method and produced up to 2 × 10<sup>6</sup> infectious units ml<sup>−1</sup>. Forty-eight hours after transfection, the supernatant was collected and used to infect Jurkat32H cells. In total, 10<sup>3</sup> cells were infected for 48 h and treated with 50 ng ml<sup>−1</sup> TRAIL. The resulting TRAIL-resistant clones were separated by single-cell sorter (MoFlo, Fort Collins, CO, USA). After amplification, cells were exposed to TRAIL for secondary screening and analysed with FACSImmun (Beckton Dickinson, Franklin lakes, NJ, USA).

(b) Rescue analysis of cDNA Total RNA was isolated from the putative positive clones. Candidate cDNAs were then amplified with SuperScript™ One-step RT–PCR system (Gibco BRL) using the library-specific primers. The resulting PCR products were purified from agarose gels and subjected to DNA sequencing analysis.

Apoptosis assay

Flow cytometry analysis was performed with fluorescence-activated cell sorter (FACS) after staining cells with 50 µg ml<sup>−1</sup> propidium iodide. Cell viability was also determined by 0.04% trypan blue exclusion assay or MTT assay. Viability of the transfectant was assessed as follows: cells grown on cover glasses were transduced with both pEGFP and effector expression plasmid for 1 day and then incubated with TRAIL for the indicated times. Cell viability was then determined based on the morphology of GFP-positive cells under a fluorescence microscope (Zeiss, Jena, Germany).

Western blot analysis

In total, 30–50 µg of cell extracts was subjected to SDS–PAGE in a buffer containing 60 mM Tris–Cl (pH 6.8), 1% SDS, 10% glycerol, and 0.5% β-mercaptoethanol, and then transferred to PVDF membranes using Semi-Dry Transfer system (Bio-Rad). The membranes were blocked with TBST buffer (20 mM Tris–Cl pH 7.5, 150 mM NaCl, 0.2% Tween-20) containing 5% nonfat dried milk, incubated for 2 h with the primary antibodies, and for an additional 2 h with HRP-conjugated secondary antibodies. Proteins were then visualised using Enhanced Chemiluminescence (ECL™, Amersham-Pharmacia).

Immunohistochemical analysis

Immunohistochemical staining was performed by the ABC method using formalin-fixed, paraffin-embedded tissue sections. Five micrometer thick tissue sections mounted on silanised slides were deparaffinised in xylene followed by sequential washes in graded ethanol to phosphate-buffered saline (PBS). The samples were pretreated for 15 min in 10% sodium citrate, pH 7.0 and endogenous peroxidase activity was blocked with 3% H<sub>2</sub>O<sub>2</sub> for 15 min. The slides were incubated with the primary antibodies and then with a biotinylated link antibody (DAKO) for 30 min followed by incubation in an avidin/biotinylated HRP solution. The samples were exposed to diamobenzidine for 6 min, counterstained with Mayer’s haematoxylin, and mounted in Permunt (Fisher Scientific, NJ, USA).

Caspase activity assays

Cells (1 × 10<sup>7</sup>) were resuspended in isolation buffer (20 mM HEPES–KOH, pH 7.6, 100 mM KCl, 0.5 mM Na-EDTA, 2 mM β-mercaptoethanol, 0.1 mM PMSF, 10 µg ml<sup>−1</sup> leupeptin, 25 µg ml<sup>−1</sup> ALLN). After incubation for 10 min at 4°C, the cells were disrupted by 20–30 strokes with a homogeniser and clarified by centrifugation for 1 h at 100 000 g. Enzymatic reactions were carried out at 37°C in reaction buffer (0.1 mM HEPES, 2 mM DT, 0.1% Chaps, 1% sucrose)
containing 20 μg protein and either 50 μM DEVD-AMC or IETD-AMC. AMC fluorescence (480 nm emission excited by illumination at 360 nm) was measured using a fluorescence microplate reader (FL-600) (Bio-TEK instrument Inc., Winooski, VT, USA).

Reverse transcription – polymerase chain reaction (RT) – PCR

Total RNA was extracted from the cells using MRC Trizol reagent (Cincinnati, OH, USA). cDNA was prepared from 1 μg of total RNA using oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (Gibco BRL), and amplified by PCR with Taq DNA polymerase. Primers used were: DR4, 5'-ctgcaggtcgtacctagctcagctgcatac-3' and 5'-cctcaatcttctgcttggcatctctctcc-3'; DR5, 5'-caggactatagcactcactggaatgacctc-3' and 5'-ctctcaatcttctgcttggcatctctctcc-3'; TRAIL, 5'-agcctgggacagacctgcgtgctgatcgtg-3' and 5'-actggctttcatggtccatgtctatcaagt-3'; β-actin, 5'-gagggaaatcgtgcgtgacat-3' and 5'-acatctgctggaaggtggaca-3'.

RESULTS

Isolation of Bcl-XL as an inhibitor of TRAIL-induced apoptosis by functional screening

To isolate genes conferring resistance to TRAIL-induced apoptosis, we have screened cDNA libraries by functional genomics.

Figure 1

Doxycycline-dependent sensitivity of Jurkat32H cells harbouring Bcl-XL to TRAIL-triggered apoptosis. (A) By cell-based functional screening of cDNA library, Jurkat32H ‘clone 2-42’ harbouring Bcl-XL and ‘clone 2-51’ harbouring FLIP were selected and isolated as showing resistance to TRAIL. Each clone was left untreated (control) or exposed to TRAIL (50 ng ml⁻¹) in the presence or absence of doxycycline (Dox, 50 ng ml⁻¹) for 48 h. Cells were then incubated with 50 μg ml⁻¹ propidium iodide and analysed by FACS. (B) Jurkat32H ‘clone 2-42’ was incubated for 48 h with increasing concentrations of TRAIL in the presence or absence of doxycycline and cell death was then evaluated by trypan blue exclusion. (C) Western blot (Bcl-XL) and RT–PCR (rescued cDNA) analyses of Jurkat32H ‘clone 2-42’ after incubation with doxycycline for 48 h.

Figure 2

Upregulation of Bcl-XL in human breast cancers. (A) Western blot analysis showing expression levels of Bcl-XL and Bcl-2 in the extracts of normal (N) and breast cancer tissues (C). Numbers indicate individual human breast cancer patient from which tissues were derived. Relative level of Bcl-2 (open box) and Bcl-XL (closed box) proteins were determined with densitometry using BIO-RAD ‘Quantity One’ image software. (B) Immunohistochemical analysis showing the expression pattern of Bcl-2 and Bcl-XL in the tissue sections prepared from human breast cancer patients. Arrowheads indicate densely stained area with anti-Bcl-XL antibody.
Jurkat32H cells, which were modified for tetracycline off-inducible expression system, were infected with a retroviral human cDNA library and exposed to TRAIL. We used 50 ng ml\(^{-1}\) of TRAIL to induce 100% of cell death after 48 h (Figure 1B). We achieved 30–40% infection as determined by doping of the library with marker retroviruses pTRA-GFP. TNF-related apoptosis-inducing ligand-resistant clones were selected out of 10\(^5\) cells by exposing to TRAIL for 48 h and isolated by FACS. DNA sequencing analysis of the cDNAs rescued from 11 TRAIL-resistant clones revealed that two of the TRAIL-resistant clones expressed Bcl-X\(_L\) and eight clones expressed FLICE-inhibitory protein (FLIP). Two of such clones, Jurkat32H ‘clone 2-42’ (Jurkat32H-Bcl-X\(_L\)) and Jurkat32H ‘clone 2-51’ (Jurkat32H-FLIP), were analysed by flow cytometry for the resistance to TRAIL (Figure 1A). Expression of the exogenous gene in the inducible expression plasmid significantly protected Jurkat cells from apoptosis, showing comparable propidium iodide uptake to the control, while treatment with doxycycline sensitised the cells to TRAIL. Quantitative determination of apoptosis showed that expression of exogenous Bcl-X\(_L\) suppressed various concentrations of TRAIL-induced apoptosis up to 200 ng ml\(^{-1}\) (Figure 1B). Doxycycline-dependent expression pattern of Bcl-X\(_L\) in Jurkat32H-Bcl-X\(_L\) cells was confirmed by Western blotting and RT–PCR analysis (Figure 1C). These results indicate that the increased expression of Bcl-X\(_L\) confers resistance to TRAIL.

**Increased expression of Bcl-X\(_L\) in human breast cancer tissues**

Based on the screening results, we examined the expression levels of Bcl-X\(_L\) and Bcl-2 in tissue extracts prepared from the human breast cancer patients (Figure 2). Western blot analysis showed that in contrast to Bcl-2, expression of Bcl-X\(_L\) was markedly increased in breast cancer tissues compared to normal; six out of seven patients showed upregulated expression pattern of Bcl-X\(_L\) (Figure 2A). Densitometric analysis indicated that Bcl-X\(_L\) was increased 4–5-fold in cancer tissues. Similarly, a significant difference in the expression patterns of Bcl-X\(_L\) and Bcl-2 was observed by immunostaining of the tumour samples using anti-Bcl-X\(_L\) and anti-Bcl-2 antibodies (Figure 2B). Tumour samples from patients with locally advanced breast cancer were more specifically stained by anti-Bcl-X\(_L\) antibody than anti-Bcl-2 antibody, consistent with the result of Western blot analysis. Note the low basal immunoreactive signal of Bcl-2 compared to consider-
able level of Bcl-XL in the tumour tissues. These results indicate that Bcl-XL is highly upregulated in the breast cancer tissues.

Different sensitivity of MDA-MB-231 and SK-BR-3, human breast tumour cell lines, to TRAIL

To explore the effects of increased expression of Bcl-XL on TRAIL-induced responses, several human breast tumour cell lines were examined for the expression levels of Bcl-2 and Bcl-XL, and their sensitivities to TRAIL (data not shown). Among them, MDA-MB-231 and SK-BR-3 cell lines were selected by their different expression levels of Bcl-XL and sensitivities to TRAIL. The death rates of MDA-MB-231 cells exposed to TRAIL were 24 and 55% at 9 and 18 h, respectively (Figure 3A). On the contrary, SK-BR-3 cells were resistant to TRAIL. Examination of expression level with Western blotting and RT-PCR followed by densitometric analysis showed that Bcl-XL was upregulated 3.8-fold in SK-BR-3 cells compared with MDA-MB-231 cells, while Bcl-2, Bax, FADD, caspase-3, caspase-8, DR4, and DR5 were not significantly different (Figure 3B–D). TRAIL was expressed in those cell lines without any detectable differences (Figure 3D, right panel).

We have then examined activation of caspase in TRAIL-resistant SK-BR-3 cells and compared it to that of TRAIL-sensitive MDA-MB-231 cells. Western blot analysis revealed that proforms of caspase-3 and -8 disappeared within 9 h of exposure to TRAIL in MDA-MB-231 cells, indicating that those caspases were proteolytically activated in the TRAIL-sensitive cells (Figure 4A). Caspase-9 appeared to be activated but less effectively. Indeed, processed and active forms of caspases, including large subunit (p20) and small subunit (p18) were detected at every time point following exposure to TRAIL (data not shown). Interestingly, procaspase-8, but not caspase-3 and -9, in the TRAIL-resistant SK-BR-3 cells was apparently reduced like MDA-MB-231 cells. Bid, a substrate of caspase-8, also decreased in both cell lines exposed to TRAIL.

Determination of enzymatic activation using fluorogenic substrate showed that TRAIL treatment induced activation of caspase-8 about 3–4-fold in both cell lines (Figure 4B). Caspase-3 was effectively activated in MDA-MB-231 cells with 5.5-fold, consistent with the result of Western blot analysis (Figure 4A), while only marginal activation of caspase-3 was observed in SK-BR-3 cells. These results led us to propose that caspase-8 was equally activated in both cell lines, whereas caspase-3 activation was suppressed in TRAIL-resistant SK-BR-3 cells.

Since Bcl-XL is known to suppress mitochondria-mediated cell death, mitochondrial membrane potential was examined with mitotracker Rhodamine 123 after exposure to TRAIL (Figure 4C). Dissipation of mitochondrial membrane potential was observed in TRAIL-sensitive MDA-MB-231 cells, but not in TRAIL-resistant SK-BR-3 cells, indicating that mitochondria-mediated death event is defective in SK-BR-3 cells expressing high level of Bcl-XL.

Attenuation of TRAIL-induced apoptosis by overexpression of Bcl-XL in TRAIL-sensitive MDA-MB-231 cells

We have then addressed whether increased expression of Bcl-XL in TRAIL-sensitive MDA-MB-231 cells suppressed TRAIL-induced apoptosis. MDA-MB-231 cells were transiently transfected with Bcl-2 or Bcl-XL expression vectors and subsequently exposed to TRAIL (Figure 5). Determination of cell viability showed that MDA-MB-231 cells expressing Bcl-XL became resistant to TRAIL; death rates decreased from 58 to 29%. Interestingly, Bcl-2 was less potent to suppress TRAIL-induced apoptosis of MDA-MB-231 cells (death rates, 58 to 49%) compared with Bcl-XL. Similar inhibitory effects of Bcl-XL and Bcl-2 were further observed in HeLa, human cervical tumour cells, and Jurkat, human lymphoma cells, permanently overexpressing Bcl-XL or Bcl-2 (Figure 6). Expression levels of Bcl-2 or Bcl-XL in these stable cell lines were 3–4-fold higher than control cells, similar ratio with the increased expression of Bcl-XL in the breast cancer tissues as shown in Figure 2. HeLa and Jurkat cells were sensitive to TRAIL, exhibiting 60–70% of death rates when exposed to TRAIL. Forced expression of Bcl-2 in those stable cells did not suppress the death rates. On the contrary, expression of Bcl-XL in HeLa or Jurkat cells substantially reduced TRAIL-induced apoptosis, indicating differential inhibitory activities of Bcl-XL and Bcl-2 in TRAIL-mediated cell death.
the expression levels of caspase-8 and FADD between SK-BR-3 and screening results. However, there were no detectable differences in TRAIL-induced apoptosis (Harper et al, 2000; Kim et al, 2000; Kischkel et al, 2000). Also FLIP, a caspase-8 interacting inhibitory protein, was recently reported to suppress TRAIL-triggered apoptosis in several tumour cell lines. Thus, the increased expression of Bcl-XL in the breast tumours and cell lines may desensitise the cells to TRAIL.

DISCUSSION

In the present study, we have screened and characterised genes inhibiting TRAIL-induced apoptosis and isolated several genes including FLIP and antiapoptotic Bcl-2 family proteins, including Bcl-XL and Mcl-1, but not Bcl-2. Unlike Bcl-2, Bcl-XL is highly upregulated in human breast cancer tissues and effective to suppress TRAIL-triggered apoptosis in several tumour cell lines. Thus, the increased expression of Bcl-XL in the breast tumours and cell lines may desensitise the cells to TRAIL.

Others and we have previously shown that caspase-8 and FADD played a critical role in TRAIL-induced apoptosis (Bodmer et al, 2000; Kim et al, 2000; Kischkel et al, 2000). Also FLIP, a caspase-8 interacting inhibitory protein, was recently reported to suppress TRAIL-induced apoptosis (Harper et al, 2001), consistent with our screening results. However, there were no detectable differences in the expression levels of caspase-8 and FADD between SK-BR-3 and MDA-MB-231 breast tumour cells showing different sensitivities to TRAIL. In addition, TRAIL-mediated activation of caspase-8 and Bid cleavage in TRAIL-resistant SK-BR-3 cells indicates that TRAIL receptor and its associated adaptor molecules such as FLIP linking to caspase-8 appeared to be functionally normal. Instead, Bcl-XL in SK-BR-3 cells is likely to be one of the components in the signalling complexes contributing to the resistance to TRAIL, probably by interfering with mitochondria-mediated apoptotic pathway, though we could not examine the sensitivity of the primary culture cells directly derived from the breast cancer tissues to TRAIL.

Though the biochemical mechanisms by which members of the Bcl-2 family of proteins inhibit apoptosis remain enigmatic, the following properties have been proposed to play a role in the modulation of apoptosis (Reed, 1997; Hengartner, 2000). First, the ability of some members to form pore or channel, through which cytochrome c and other intermembrane proteins escape with low selective permeability, similar to some pore-forming bacterial toxins (Minn et al, 1997). Second, the ability of the different members of this family to function as docking proteins able to bind each other to form homo- or heterodimers as well bind to other proteins (Sato et al, 1994; Sedlak et al, 1995). Thus, the ratio between proapoptotic and antiapoptotic molecules in a particular cell may then determine the response to an apoptotic stimulus. However, there was no detectable difference in the expression level of Bax, a proapoptotic molecule interacting with Bcl-2 and Bcl-XL, between cell lines used in this study.

Interestingly, Bcl-2 did not show comparable antiapoptotic activity on TRAIL-mediated apoptosis, indicating that the death signalling activated by TRAIL was not susceptible to the inhibition mediated by Bcl-2 in the tumour cells we have examined. However, the ability of Bcl-2 to suppress apoptosis triggered by TRAIL is controversial. Human glioma and prostate cancer cells lost their sensitivity to TRAIL by overexpressing Bcl-2 (Rieger et al, 1998; Munshi et al, 2001; Rokhlin et al, 2001). In contrast, other groups have shown that Bcl-2-transfected 8226 and ARP-1 myeloma cells still remained sensitive to TRAIL (Gazitt, 1999a, b) and that Bcl-2 failed to block cytochrome c release after exposure to TRAIL (Keogh et al, 2000; Walczak et al, 2000). Although the discrepancy between these observations is not clearly resolved, it might be arisen from difference in cell types or in the relative expression level of Bcl-2 family proteins including Bcl-2 itself.

Although function of Bcl-2 family proteins looks similar, increasing numbers of evidences have suggested that prosurvival activities of Bcl-2 and Bcl-XL are differently regulated. Bcl-2 and

Figure 5  Ectopic expression of Bcl-XL but not Bcl-2, rescued MDA-MB-231 cells from TRAIL-induced apoptosis. MDA-MB-231 cells were transiently transfected with pEGFP and either pcDNA3 (control), pBcl-2, or pBcl-XL. pEGFP was included in every transfection reactions with a ratio of 1:3. After 24 h, cells were incubated with TRAIL for 18 h and death rates were determined based on the morphology of GFP-positive cells under a fluorescence microscope. Cell extracts prepared from each transfectant were subjected to Western blot analysis.

Figure 6  Differential effect of Bcl-XL and Bcl-2 on TRAIL-induced apoptosis. HeLa and Jurkat cells permanently expressing Bcl-XL or Bcl-2 were exposed to TRAIL (80 ng ml⁻¹) for the indicated times and cell viability was determined by trypan blue and MTT assays. Expression levels of Bcl-XL or Bcl-2 in the respective cell line were examined by Western blot analysis.
Bcl-X1 may function at distinct sites (Chinnainayi et al., 1997; El-Assaad et al., 1998). It is believed that death receptor-induced apoptotic pathway generally bypasses the Bcl-2-inhibitable steps, whereas Bcl-2 protects against diverse cytotoxic insults, for example, γ- and ultraviolet-irradiation, cytokine withdrawal, dexamethasone, and cytotoxic drugs (Cory, 1995; Strasser et al., 1995; Yang and Korsmeyer, 1996; Chao and Korsmeyer, 1998). In contrast, Bcl-X1 is thought to be more potent to suppress death receptor-induced cell death pathway (Fernandez et al., 2000). Thus, cancer cells may increase expression of Bcl-X1 rather than Bcl-2 to be resistant to ligand-mediated cytotoxic stimuli including TRAIL.

Taken together, we propose here that the increased expression of Bcl-X1 observed in the human breast tumours desensitises tumourigenic cells to apoptosis triggered by TRAIL.

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