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Inhibition of apoptosis by the actin-regulatory protein gelsolin

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Abstract

Gelsolin is an actin-regulatory protein that modulates actin assembly and disassembly, and is believed to regulate cell motility invivo through modulation of the actin network. In addition to its actin-regulatory function, gelsolin has also been proposed to affect cell growth. Our present experiments have tested the possible involvement of gelsolin in the regulation of apoptosis, which is significantly affected by growth. When overexpressed in Jurkat cells, gelsolin strongly inhibited apoptosis induced by anti-Fas antibody, C2-ceramide or dexamethasone, without changing the F-actin morphology or the levels of Fas or Bcl-2 family proteins. Upon the induction of apoptosis, an increase in CPP32(-like) protease activity was observed in the control vector transfectants, while it was strongly suppressed in the gelsolin transfectants. Pro-CPP32 protein, an inactive form of CPP32 protease, remained uncleaved by anti-Fas treatment in the gelsolin transfectants, indicating that gelsolin blocks upstream of this protease. The tetrapeptide inhibitor of CPP32(-like) proteases strongly inhibited Fas-mediated apoptosis, but only partially suppressed both C2-ceramide- and dexamethasone-induced apoptosis. These data suggest that the critical target responsible for the execution of apoptosis may exist upstream of CPP32(-like) proteases in Jurkat cells and that gelsolin acts on this target to inhibit the apoptotic cell death program. Keywords: apoptosis/CPP32/Fas/gelsolin/Jurkat cells

Introduction

Apoptosis, or genetically regulated cell death, is crucial for normal development and tissue remodeling in multicellular organisms (Wyllie etal., 1980). In recent years, much effort has been made to identify the components which regulate apoptosis, and it is now recognized that members of the interleukin-1-converting enzyme (ICE) family of cysteine proteases act as `executionersÅ in the cell death process (Martin and Green, 1995). CPP32 (also called Yama or apopain) is a major protease in this growing family, known to be involved and probably essential in various types of apoptosis

(Fernandes-Alnemri etal., 1994; Nicholson etal., 1995; Tewari etal., 1995). On the other hand, the Bcl-2 proto-oncogene product (Bakhshi etal., 1985; Cleary and Sklar, 1985; Tsujimoto etal., 1985) and the related protein Bcl-xL (Boise etal., 1993) have been shown to exert an apoptosis-inhibitory function (Vaux etal., 1988; Tsujimoto etal., 1989; Nunez etal., 1990). Two viral anti-apoptotic proteins, CrmA and p35, have also been shown to suppress diverse forms of apoptosis (Clem etal., 1991; Ray etal., 1992). Recent studies in which these apoptosis-inhibitory proteins were overexpressed, or where tetrapeptide inhibitors specific for each ICE family protease were used, have generated data helpful for the interpretation of the molecular mechanisms of apoptosis. Thus, the finding of any new inhibitor able to suppress various forms of apoptosis will benefit further study that seeks to understand the machinery of apoptosis.

Gelsolin is an actin-regulatory protein, first isolated from rabbit lung macrophages as a modulator of the cytoplasmic actin gel-sol transformation (Yin and Stossel, 1979). Gelsolin can sever actin filaments and cap the fast-growing ends of the filaments invitro; this promotes actin disassembly. Gelsolin also has the ability to nucleate actin polymerization. These functions are activated by Ca2+ and inhibited by polyphosphoinositides (PPIs) (Yin and Stossel, 1980; Kurth and Bryan, 1984; Janmey and Stossel, 1987). Such observations have led to the view that gelsolin regulates actin reorganization in response to changes in the concentrations of Ca2+ and PPIs in living cells (Yin, 1988).

The overexpression of gelsolin in NIH 3T3 fibroblasts results in their enhanced motility, indicating that the regulation of cell motility is one of the roles of gelsolin invivo (Cunningham etal., 1991). A recent invivo study has supported this by demonstrating blunted leukocyte motility in transgenic gelsolin-null mice (Witke etal., 1995). Further evidence supports the idea that gelsolin is also able to regulate cell growth: a mouse gelsolin gene with a point mutation revealed a tumor suppressive potential against H-ras oncogene-transformed NIH 3T3 cells (Mullauer etal., 1993; Fujita etal., 1995). In addition, authentic gelsolin transfectants of a human bladder cancer cell line, UMUC-2, greatly reduced colony-forming ability and tumorigenicity invivo (Tanaka etal., 1995).

Several reports have provided evidence that proteins which fulfill apoptosis-regulatory functions may simultaneously possess the ability to regulate cell proliferation, as is the case with Bcl-2 (Borner, 1996; Mazel etal., 1996), Ras (Trent etal., 1996) or p53 (Lassus etal., 1996). We therefore tested the possibility that gelsolin may have an apoptosis-regulatory function in the Jurkat cell model system. By using three different apoptotic stimuli, all of which activate CPP32(-like) proteases, we found strong evidence that gelsolin acts as an apoptosis inhibitor upstream of CPP32(-like) proteases.

Results

Establishment of Jurkat cell lines that overexpress gelsolin To investigate the effects of overexpressed gelsolin on apoptosis, we established stable transfectants of Jurkat

cells overexpressing gelsolin, and designated them JGF-1-8. Western blot analysis showed two to three times higher amounts of gelsolin in JGF clones than in either the parental cells (Figure 1A) or the control vector transfectants (designated JNF-1-5, data not shown), with no difference in the amounts of actin (Figure 1A). Phase-contrast microscopy showed that overexpressed gelsolin had induced no signi ficant difference in cellular morphology (data not shown), nor were the distribution or amounts of F-actin affected by gelsolin, as assessed respectively by confocal laser microscopy (Figure 1B) and fluorescence-activated cell sorting (FACS) analyses (data not shown) of cells stained with rhodamine-phalloidin. Two representative clones of each genotype (JNF-2, JNF-5, JGF-5 and JGF-7) were used for further studies.

Inhibition of apoptosis by overexpressed gelsolin To induce apoptosis, cells were treated with anti-Fas antibody (Itoh etal., 1993), C2-ceramide (C2-Cer) (Obeid etal., 1993) or dexamethasone (Dex) (Cohen and Duke, 1984), and cell viability was assessed by trypan blue exclusion. JGF-5 and JGF-7 cells showed remarkable resistance to all apoptosis-inducing treatments compared with both parental Jurkat and JNF-2 cells (Figure 2A). The extent of apoptotic cell death depended upon the concentrations of anti-Fas antibody; while JNF-2 cells were almost completely killed by anti-Fas antibody at 100ng/ml for 24h, 65% of JGF-5 cells were still viable at a 10-fold higher concentration (Figure 2B). The absence of internucleosomal DNA fragmentation in JGF-5 cells treated with anti-Fas antibody (Figure 2C) or with either C2-Cer or Dex (Figure 2D) confirmed gelsolinÅs anti-apoptotic activity.

Although these stimuli did not induce significant cell death in JGF cells, growth inhibition was observed commonly with each stimulus (data not shown). We therefore investigated the cell cycle profiles of cells treated with the apoptotic stimuli. With no treatment, JNF-2 and JGF-5 cells showed similar cell cycle profiles [Figure 3, (-)]. When cell death was induced using any one of three apoptotic stimuli, sub-G0/G1 apoptotic populations became apparent in JNF-2 cells, but not in JGF-5 cells (Figure 3). Interestingly, JGF-5 cells showed quite different cell cycle profiles with each stimulus: G2/M arrest with C2-Cer, G1 accumulation with Dex and a slight decrease in S phase with anti-Fas treatment. This result suggests that the three stimuli exert different biological effects on Jurkat cells, although cell death is a common consequence. Gelsolin therefore appears to impinge upon a mechanism central to apoptosis induced by clearly distinct stimuli.

Gelsolin alters neither Fas expression nor the amounts of Bcl-2 family proteins FACS analysis revealed that gelsolin overexpression did not alter Fas expression on the surface of Jurkat cells (Figure 4A), and Western blot analysis showed no differences in the amounts of Bcl-2 and Bcl-xL between parental Jurkat cells and the transfected lines (Figure 4B). Bax protein, another member of the Bcl-2 family, has been shown to dimerize with either Bcl-2 or Bcl-xL and promotes apoptosis (Oltvai etal., 1993; Sedlak etal., 1995), and the level of this protein was also unaltered (Figure 4B). These results indicate that gelsolin exerts its anti-apoptotic activity neither through the reduction of Fas antigen nor by changing the levels of Bcl-2 family proteins.

Gelsolin inhibits the activation of CPP32(-like) proteases Recent studies have shown that members of the ICE family of proteases play a central role in apoptosis (Miura

etal., 1993; Wang etal., 1994; Faucheu etal., 1995; Fernandes-Alnemri etal., 1995; Munday etal., 1995). To examine the possible involvement of gelsolin in the regulation of the activity of ICE family proteases, we measured the activity of ICE(-like) proteases and CPP32(-like) proteases using peptide substrates in cells treated with anti-Fas antibody, C2-Cer or Dex, with or without overexpressed gelsolin. We were unable to find any ICE(-like) protease activity in apoptotic Jurkat cells, nor could we detect either ice mRNA or ICE protein in these cells (data not shown), consistent with previous observations (Schlegel etal., 1996). In contrast, CPP32(-like) protease activity in control JNF-2 cells was clearly elevated with all inducers of apoptosis (Figure 5A), and the increase preceded the appearance of apoptotic cells (Figure 2A). In JGF-5 cells, however, the induction of CPP32(-like) protease activity was strongly suppressed (Figure 5A), suggesting that gelsolin blocks apoptosis, in part, by inhibiting the usual increase in CPP32(-like) protease activity seen in response to inducers.

CPP32 protease is synthesized as a 32kDa inactive precursor (pro-CPP32) which is proteolytically cleaved to produce a mature enzyme composed of 17and 12kDa subunits (Fernandes-Alnemri etal., 1994; Nicholson etal., 1995; Tewari etal., 1995). To determine whether gelsolin inhibits CPP32(-like) proteases which have already been activated, or prevents the initial activation of these proteases, we examined the cleavage of the pro-CPP32 protein in response to apoptosis-inducing treatments. The results in Figure 5B indicate that the pro-CPP32 protein disappears upon the induction of apoptosis by anti-Fas antibody in parental Jurkat and JNF-2 cells, reflecting the proteolytic cleavage of pro-CPP32 generating active CPP32 protease. In contrast, the pro-CPP32 protein remains uncleaved in identically treated JGF-5 cells. Similar results were obtained with C2-Cer or Dex treatment (data not shown). Taken together, these results strongly suggest that gelsolin suppresses a common upstream step in the pathway leading to the activation of CPP32(-like) proteases in response to diverse stimuli, rather than inhibiting these proteases directly.

To determine if ICE family proteases are required for the apoptosis of Jurkat cells, we next treated cells with anti-Fas antibody, with C2-Cer or with Dex in the presence of Ac-DEVD-CHO, a CPP32/apopain-specific inhibitor (Nicholson etal., 1995), or of Ac-YVAD-CHO, an ICE-specific inhibitor (Thornberry etal., 1992). As shown in Figure 6, Ac-DEVD-CHO effectively inhibited Fas-mediated apoptosis in Jurkat cells at 0.1mM while Ac-YVAD-CHO had no inhibitory effect at concentrations as high as 1mM, suggesting that CPP32(-like) proteases, but not ICE(-like) proteases, are essential for Fas-mediated apoptosis in Jurkat cells. This is consistent with previous observations (Schlegel etal., 1996) and with our observation that ICE protease is not expressed in this model system. However, Ac-DEVD-CHO had only a partially inhibitory effect on C2-Cer- or Dex-induced apoptosis in these cells (Figure 6). This suggests that CPP32(-like) proteases are also involved in C2-Cer- or Dex-induced apoptosis of Jurkat cells, but that they are only partially requisite in response to these agents. Thus, other ICE family proteases or other key steps, which are insensitive to both of the inhibitors above, but can be inhibited efficiently by gelsolin overexpression, may act as an essential mediator of these forms of apoptosis. These experiments are consistent with gelsolin inhibiting apoptosis by blocking a target upstream of the CPP32(-like) proteases which is essential for, and common among, the pathways of apoptosis induced by markedly different stimuli.

Discussion

These studies have shown that when overexpressed in Jurkat cells, gelsolin inhibits apoptosis induced by three different stimuli. A similar anti-apoptotic activity of gelsolin has been observed in mouse fibroblast NIH 3T3 cells and other human tumor cell lines with several different apoptotic stimuli (N.Sakai, M.Ohtsu, H.Fujita and N.Kuzumaki, in preparation), indicating that this activity is not restricted to Jurkat cells. Several possible mechanisms may explain this inhibitory activity, including modulation of the actin network and inhibition of certain ICE family proteases.

Since gelsolin has actin-regulatory functions, modulation of the actin network might be responsible for the inhibition of apoptosis. However, Western blot analysis showed that the total amount of cellular actin was not altered in response to gelsolin overexpression. Moreover, F-actin analysis with rhodamine-phalloidin staining revealed no apparent change in the distribution or amounts of F-actin in the gelsolin transfectants. These results suggest that obvious alterations in the actin network have not occurred in response to gelsolin overexpression, and therefore did not contribute to the apoptosis-inhibitory effect of gelsolin. Since monomeric (G-) actin has been shown to inhibit DNase I activity (Peitsch etal., 1993), an increase of G-actin, although undetectable in our study, might inhibit DNase I and consequently suppress DNA fragmentation. However, since DNA fragmentation is probably a late event in the apoptotic pathway, inhibition of DNase I is unlikely to explain the blockage of CPP32(-like) protease activation and apoptosis observed in the gelsolin transfectants.

One study has reported that anti-CD3-induced growth arrest in Jurkat cells accompanies rapid and major actin reorganization, and that treatment with cytochalasin D, an inhibitor of actin polymerization, not only prevents the change in cell shape but also blocks anti-CD3-mediated signal transduction leading to growth arrest (Parsey and Lewis, 1993). Although this suggests that modulation of actin reorganization may also block signal transduction from the Fas antigen, we were unable to find any early changes in F-actin morphology in Jurkat cells treated with anti-Fas antibody (data not shown). In addition, inhibition of actin polymerization by treatment with cytochalasin D did not prevent Fas-induced apoptosis (data not shown), suggesting that signaling along this pathway does not require actin reorganization. Additional experiments with gelsolin mutants which lack various actin-regulatory functions are now underway to address this possibility more completely.

The anti-apoptotic proteins CrmA and p35 recently have been shown to inhibit apoptosis by directly inhibiting specific ICE family protease(s) (Rabizadeh etal., 1993; Bump etal., 1995; Tewari and Dixit, 1995), most likely by functioning as substrates for, and as competitive inhibitors of, these enzymes (Xue and Horvitz, 1995). Gelsolin itself might also be a substrate, and thus an inhibitor of certain ICE family proteases. We can find two potential cleavage sites for CPP32 in the amino acid sequence of human cytoplasmic gelsolin (Kwiatkowski etal., 1986). These sequences are DQTD352G and SEPD588G, which respectively resemble the cleavage sites DQMDG in p35 (Bertin etal., 1996) and SEPDS in the sterol-regulatory element-binding protein (SREBP)-1 (Wang etal., 1996). Recently,

FLICE/MACH1/Mch-5 has been identified as a new member of the ICE family of proteases which possesses dual functions. One function is to interact with Fas antigen intracellularly, and another is to operate as an ICE family protease at the apex of the apoptotic protease cascade (Boldin etal., 1996; Muzio etal., 1996). It thus seems likely that the cross-linking of Fas antigen leads to the activation of this protease which in turn activates the intermediate ICE family proteases, and these proteases activate CPP32(-like) proteases which are probably the final effectors of the protease cascade (Fraser and Evan, 1996). More recently, Mch-4, a novel protease structurally related to FLICE/MACH1/Mch-5, has been cloned from a Jurkat cDNA library and shown to have the ability to cleave pro-CPP32 protease (Fernandes-Alnemri etal., 1996). In this relatively simple pathway of Fas-mediated apoptosis, it is tempting to speculate, dueto the very potent inhibition of apoptosis by gelsolin, thatit may inhibit additional ICE family proteases (including FLICE/MACH1/Mch-5, Mch-4) upstream of the CPP32(-like) proteases.

Ceramide has also been proposed to be a regulator of apoptosis by virtue of serving as a novel lipid second messenger in several signal transduction pathways, including one culminating in apoptosis (Hannun and Obeid, 1995), and recent reports suggest that ceramide is an endogenous mediator of Fas-mediated apoptosis (Cifone etal., 1993; Tepper etal., 1995). In this study, however, we present evidence that C2-Cer acts as an alternative apoptotic stimulator rather than as a mediator of Fas-induced apoptosis. C2-Cer treatment induced apoptosis and the activation of CPP32(-like) proteases in Jurkat cells, both of which were inhibited by overexpressed gelsolin. On the other hand, the inhibitor of CPP32(-like) proteases, Ac-DEVD-CHO, was revealed to be partially inhibitory to C2-Cer-induced apoptosis, suggesting that C2-Cer stimulates an additional cell death pathway distinct from CPP32(-like) protease activation. Additionally, the biological effects of C2-Cer are probably not the same as those of increased endogenous ceramide (Pronk etal., 1996), consistent with our observations. Since C2-Cer has been shown to activate a stress-activated protein kinase (SAPK/JNK), which results in c-Jun activation-dependent apoptosis (Verheij etal., 1996), SAPK/JNK activation may therefore transduce the C2-Cer-generated signal via this distinct signal transduction pathway.

The mechanism of Dex-induced apoptosis remains largely unknown. Since the glucocorticoid receptor (GR) is required for Dex-induced apoptosis, GR-mediated induction of `lysis genesÅ or repression of `survival genesÅ is thought to be responsible for apoptosis (Helmberg etal., 1995). However, the identity and nature of these lysis and survival genes remain obscure, and it is unknown how the modification of the expression of such genes leads to CPP32(-like) protease activation. Based on our observations that Dex treatment arrests cells in phases of the cell cycle that are distinct from those seen in response to the binding of Fas antigen or the addition of C2-Cer, and the fact that GR activation results in rapid binding of the receptor to DNA, it also appears that Dex may induce apoptosis by a discrete pathway. If this is the case, gelsolin must therefore block the effects of the Fas-, C2-Cer- and Dex-induced pathways which all result in apoptosis in this model system. Thus, the identification of the target(s) of gelsolin, which appear to be shared by different apoptotic pathways, should make substantial contributions to understanding the global apoptotic machinery.

The data presented here, that at relatively low intracellular levels gelsolin can act as a potent inhibitor of apoptosis induced by different agents, provide new insights into the involvement of actin-regulatory proteins in the modulation of apoptosis. Although we have presented evidence consistent with the idea that gelsolin acts as an inhibitor of apoptotic proteases, further experiments are underway to determine the precise mechanism responsible for gelsolinÅs anti-apoptotic activity.

Materials and methods

Cell culture and transfection

Jurkat cells, a lymphoblastoid T-cell line, were maintained in RPMI-1640 medium containing 10% fetal bovine serum. Transfections were carried out with either a human gelsolin expression plasmid LKCG (Cunningham etal., 1991) or an empty vector LK444 (Gunning etal., 1987) using Lipofectin. Stable transfectants were selected in the presence of 1mg/ml of G418 (Geneticin) to obtain the gelsolin transfectants JGF-1-8 and the control transfectants JNF-1-5. The reagents were all purchased from Gibco BRL.

Western blot analysis

Cells were extracted in RIPA buffer (10mM Tris-HCl, pH 8.0,150mM NaCl, 1% NP-40, 0.1% deoxycholate, 1mM EDTA, 10g/ml aprotinin and 1mM phenylmethylsulfonyl fl uoride). The indicated amounts of protein were separated on 10or 12% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane. The following antibodies were used as primary antibody; anti-human gelsolin monoclonal antibody (GS-2C4, BioMaker), anti-human actin monoclonal antibody (Bohringer Mannheim), anti-Bcl-2 polyclonal rabbit antibody raised against GST-human Bcl-2 fusion protein, anti-human Bcl-xL polyclonal rabbit antibody (Santa Cruz Biotech.), anti-human Bax polyclonal rabbit antibody (Santa Cruz Biotech.) and anti-human CPP32 monoclonal antibody (Transduction Laboratories). Either peroxidase-conjugated goat F(ab)2 anti-mouse IgG+M (Jackson ImmunoResearch Lab.) or peroxidase-conjugated goat F(ab)2 anti-rabbit IgG (TAGO) was used as secondary antibody. Detection of the bound antibodies was performed using the ECL system (Amersham).

Fluorescence microscopy and flow cytometry

F-actin staining of cells was performed as previously described (Phatak etal., 1988), using rhodamine-phalloidin (Molecular Probes, Inc.). Stained cells were examined and photographed with a Bio-Rad MRC1024 Laser Scanning Confocal Imaging System. For cell cycle analysis, ethanol-fixed cells were stained with propidium iodide (100g/ml) in the presence of RNase A, then analyzed with a FACScan flow cytometer (Beckton Dickinson). Amounts of surface Fas antigen were determined by FACS analysis using anti-Fas antibody (clone CH-11, Medical and Biological Laboratories) as described (Tepper etal., 1995).

Analysis of apoptosis

Cell viability was determined by trypan blue exclusion, and the existence of apoptotic cells was also confirmed by the appearance of sub-G0/G1 peak fractions in cell cycle analysis. To induce apoptosis, cells were seeded at 1Å~106 /ml into 24-well plates and treated with anti-Fas antibody (CH-11, 100ng/ml), C2-Cer (100M) or Dex (Wako,

200g/ml). Cell viability was assessed at the indicated times. For the assessment of dose response with anti-Fas antibody, cells were treated with the antibody at the indicated concentrations, and cell viability was examined after 24h incubation. DNA fragmentation assay was performed as previously described (Itoh etal., 1993), with the same culture conditions as above. For the experiments with ICE family protease inhibitors, cells were seeded at 1Å~106 /ml into 96-well plates and treated as above in the absence or presence of 0.1or 1mM tetrapeptide CPP32/apopain inhibitor Ac-DEVD-CHO (Peptide Institute) (Nicholson etal., 1995), or 1mM ICE inhibitor Ac-YVAD-CHO (Peptide Institute) (Thornberry etal., 1992). The corresponding amounts of vesicles (ethanol or/and dimethylsulfoxide) were added to control wells, with no significant effect on cell viability. After 24h (anti-Fas or C2-Cer) or 48h (Dex) incubation, cell viability was assessed. The inhibitors were present from 1h prior to and throughout the experiments.

Measurement of ICE(-like) and CPP32(-like) protease activity
Cells were treated in 24-well plates as described above and harvested at the indicated times. After washing with phosphate-buffered saline, cells were incubated in 50mM Tris-HCl (pH 7.4), 1mM EDTA, 10mM EGTA containing 10M digitonin at 37ÅC for 10min. After clarification by centrifugation at 15000r.p.m. for 10 min, cleared lysate (60g of protein) was incubated at 37ÅC with 50M DEVD-MCA (substrate for apopain, Peptide Institute) (Nicholson etal., 1995) for 30min or YVAD-MCA (substrate for ICE, Peptide Institute) (Thornberry etal., 1992) for 60min (data not shown for YVAD-MCA). The amounts of released 7-amino-4-methylcoumarin (AMC) were measured with a spectrofluorometer (Perkin-Elmer LS50B) with excitation at 380nm and emission at 460nm. One unit was defined as the amount of enzyme required to release 1pmol of AMC per min at 37ÅC.

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Figure legends:

Fig. 1. Western blot analysis and F-actin morphology of the parental Jurkat cell line and its transfectants. (A) Amounts of gelsolin and actin in parental Jurkat and JGF-1-8 cells examined by Western blot analysis. Proteins (20g) were separated on 10% SDS-PAGE and detected as described in Materials and methods. (B) F-actin staining of JNF-2 and JGF-5 cells. Shown are representative cells of each clone. Scale bars represent 10m.

Fig. 2. Inhibition of apoptosis by overexpressed gelsolin. (A) Time course analysis of the viability of parental Jurkat, JNF-2, JGF-5 and JGF-7 cells treated with anti-Fas antibody, C2-Cer or Dex. Results are shown as meansÅ}SD of values obtained from three independent experiments. (B) Dose dependence of viability (24h) of JNF-2 and JGF-5 cells on the concentration of anti-Fas antibody. Results are shown as mean values from two independent experiments. (C and D) DNA fragmentation assay. DNA was extracted from JNF-2 or JGF-5 cells treated with each stimulus for the indicated periods and subjected to a 2% agarose gel electrophoresis. M, DNA size marker (X174-HaeIII digest).

Fig. 3. Cell cycle profiles of control or gelsolin transfectants treated with apoptotic stimuli. JNF-2 or JGF-5 cells were treated as in Figure 2A, and harvested at 24h (C2-Cer and anti-Fas) or 36h (Dex) for cell cycle analysis. A sub-G0/G1 fraction representing the apoptotic cell population is shown as `ApoÅ. JGF-7 cells exhibited almost identical profiles to JGF-5 cells.

Fig. 4. Expression of Fas antigen and Bcl-2 family proteins in the parental Jurkat cell line and its transfectants. (A) Cell surface expression of Fas antigen in parental Jurkat, JNF-2 and JGF-5 cells. JNF-5 and two other JGF clones showed almost identical results. (B)Amounts of Bcl-2, Bcl-xL and Bax proteins in parental Jurkat, JNF-2, JNF-5, JGF-5 and JGF-7 cells. Proteins (50g) from exponentially growing cells were separated on 12% SDS-PAGE and analyzed as in Materials and methods.

Fig. 5. Blocked activation of CPP32(-like) proteases by gelsolin. (A)Inhibition of the increase in CPP32(-like) protease activity in JGF-5 cells. Results are shown as meansÅ}SD of values obtained from three independent experiments. (B) Western blot analysis of pro-CPP32 proteins in parental Jurkat, JNF-2 and JGF-5 cells with anti-Fas treatment. Cells were treated with (1or 2h) or without (0h) anti-Fas antibody (500ng/ml), and proteins (40 g) were separated on 12% SDS-PAGE. Pro-CPP32 proteins were detected as described in Materials and methods.

Fig. 6. Effects of tetrapeptide inhibitors for ICE(-like) or CPP32(-like) proteases on apoptosis in untransfected Jurkat cells. Results are shown as meansÅ}SD of values obtained from four independent experiments.

A

B

JNF-2

JGF-5

A
Anti-Fas
C2-Cer
Dex
Cell viability (%)
100
80
60
40
20
0
0
6
12
18
24
36
48
Time (h)
Jurkat
JNF-2
JGF-5
JGF-7

B
Cell viability (%)
100
10
0.1
1
10
100
1000
Anti-Fas Ab (ng/ml)
JNF-2
JGF-5

C
Anti-Fas Ab
JNF-2
JGF-5
M
0
3
6
9
0
3
6
9
(h)

D
C2-Cer
Dex
JNF-2
JGF-5
JNF-2
JGF-5
M
0 2 6
0 2 6
4 8
4 8
(h)

JNF-2
JGF-5
G0/G1
Apo
G2/M
S
(-)
C2-Cer
Dex
Anti-Fas
Relative Cell Number
Fluorescence Intensity
150
0
0
200
400
600
800
1000

A

Jurkat
JNF-2
JGF-5
Relative Cell Number
350
0
400
0
400
0
10^0
10^1
10^2
10^3
Fluorescence Intensity

B
Jurkat
JNF
JGF
2
5
5
7
Bcl-2
Bcl-xL
Bax

A

Anti-Fas
C2-Cer
Dex
CPP32(-like) activity (units/mg prot.)
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6
2000
1500
1000
500
0
0 1 2 3 4 5 6
1200
1000
800
600
400
200
0
0 6 12 18 24
Time (h)
JNF-2
JGF-5

B
Jurkat
JNF-2
JGF-5
0 1 2 0 1 2 0 1 2 (h)
pro-CPP32

Cell viability (%)
100
80
60
40
20
0
Anti-Fas
C2-Cer
Dex
DMSO
Ac-DEVD-CHO 0.1 mM
Ac-DEVD-CHO 1 mM
Ac-YVAD-CHO 1 mM