

Molecular Cloning and Characterization of a Novel Caspase-3 Variant That Attenuates Apoptosis Induced by Proteasome Inhibition

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Caspase-3 plays an important role in programmed cell death as an execution-phase caspase in degradation of many substrate proteins. We identified a naturally occurring short caspase-3 variant (caspase-3s) from a human carcinoma cell line that is resulted from alternative mRNA splicing. Analysis of nucleotide sequence reveals a deletion of the exon 6 in this variant that resulted in an altered reading frame in the C-terminus, leading to an altered amino acid sequence and a truncated protein. Caspase-3s shares the same amino acid sequence as caspase-3 in the N-terminus containing the prodomain and the majority of the large subunit. The variant is 95 amino acid residues shorter at the C-terminus and is missing the conserved QACRG sequence in the catalytic site. Caspase-3 and caspase-3s are coexpressed in all human tissues examined. Several cancer cell lines also show coexpression of caspase-3 and caspase-3s, both at the mRNA and protein levels. Overexpression of caspase-3s in 293 cells is more resistant to apoptosis induced by proteasome inhibition. Furthermore, we identified that proteasome inhibition stabilized the level of caspase-3s. © 2001 Academic Press

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The integrity and normal function of tissues and organs require the presence of a constant number of functional cells. While tissues and organs continually renew themselves by cell proliferation, cells that are

Abbreviations used: caspase-3s, caspase-3 short variant; ELISA, enzyme-linked immunosorbent assay; IGAL, proteasome inhibitor I (Z-Ile-Glu(otBu)-Ala-Leu-CHO); LDH, lactate dehydrogenase; PARP, poly(ADP)ribose polymerase.

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not functioning properly and those that have lost the necessity of existence during development are eliminated through an evolutionally conserved mechanism, called apoptosis, also known as programmed cell death. Apoptosis plays an important role in maintaining the normal function of various tissues and organs in mammalian species. Disruption of this mechanism can have a profound impact on the functionality of certain tissues and has been implicated in many human diseases. For example, inappropriate activation of apoptosis that leads to neuronal cell death has been implicated in several neurodegenerative diseases including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS) (1–3). On the other hand, failure to eliminate damaged cells through apoptosis can lead to neoplastic cell growth and has been thought to be related to the development of several cancers (4).

The major players of apoptosis in mammalian cells are a group of caspases, a family of structurally related cysteine proteases that contain a conserved QACXG (where X is R, Q or G) pentapeptide active site motif (5). These caspases can be divided into different subgroups according to their role in apoptosis (6). One subgroup of caspases, which include caspase -8, -9, and -10, is upstream of the activation cascade. These caspases are activated by apoptotic signals. The activation of these caspases lead to the activation of executioner caspases, another subgroup of caspases targeting substrates that are directly related to cell death. These executioner caspases include caspase-3, -6, and -7. Cleavage of the substrates, which include lamins, DNA-PK, PARP, α - and β fodrin, is responsible for the morphological appearance of apoptosis including DNA fragmentation, chromatin condensation, membrane blebbing, and cell shrinkage (7).

Among all the caspases identified, caspase-3, one of the terminal caspases, plays an important role in the execution of apoptosis (6). Caspase-3 is essential for the normal development of the central nervous system.

Caspase-3 knock-out mice exhibited decreased apoptotic cell death in the brain and died prematurely (8). Caspase-3 is synthesized as a precursor and is activated by sequential caspase-8/9 processing and autolysis that generates a large and small subunit (9). The active form of caspase-3 is a homodimer of heterodimers containing the large subunit, p17 and small subunit, p12 (10–11). Caspase-3 recognizes a tetrapeptide sequence DXXD with absolute requirement of Asp in the P1 position (10, 12). In our efforts to clone caspase-3 by RT-PCR for other studies, we recognized an additional PCR product in addition to the predicted caspase-3 cDNA. We hypothesized that the additional PCR product might be a variant of caspase-3. In this study, we reported the cloning and characterization of a shorter splice-variant of caspase-3 (caspase-3s) from a human colon carcinoma cell line, the DLD-1 cells. Caspase-3s expressed at a low level in multiple human tissues and cell lines. Transfection of caspase-3s expressing constructs into 293 cells resulted in a low expression of caspase-3s protein that can be accumulated by proteasome inhibitor treatment and protected cells from apoptosis induced by proteasome inhibitor.

MATERIALS AND METHODS

Cell cultures. Cells that used in the study were maintained in Dulbecco's MEM (Life Technologies/BRL) supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 units/ml) at 37°C with 5% CO₂ in air. Cells were routinely subcultured with trypsin (0.25%)/EDTA (1 mM) when they reached 80 to 90% confluence.

RNA isolation. Total cellular RNA was isolated with TRIzol Reagent (GIBCO BRL) following the protocol specified by the supplier. Briefly, cells were lysed directly in the culture dish by the addition of TRIzol Reagent (1 ml/100-mm dish). Cell lysates were collected in a fresh tube and pipetted a few times to solubilize the RNA, followed by addition of 0.2 ml of chloroform. The tubes were shaken vigorously for 15 s and kept at room temperature for 5 min. The samples were subsequently centrifuged for 15 min at 14,000 rpm (4°C). The resulting aqueous phase was transferred to a fresh tube, and 500 μ l of isopropanol was added. The samples then were incubated in room temperature for 10 min, followed by centrifugation for 15 min at 14,000 rpm (4°C). The RNA pellet was washed with 70% ethanol, dissolved in ddH₂O, and subjected to reverse transcription-polymerase chain reaction (RT-PCR).

RT-PCR and molecular cloning of caspase-3 and caspase-3s cDNA. RT-PCR was carried out as previously described (13). Briefly, 5–10 μ g of total RNA was reverse transcribed into first strand cDNA in a reaction mixture containing 1 μ g of oligo(dT) (Pharmacia), 40 units of RNasin (Promega), 45 units of AMV reverse transcriptase (Seikagaku America Inc.), and 1 \times RT buffer (100 mM Tris, 80 mM KCl, 10 mM MgCl₂, 1 mM dNTP) at 42°C for 90 min. Sodium hydroxide was added to samples to a final concentration of 0.5 N and incubated at 70°C for 30 min. DNA was precipitated in the presence of 0.1 volume of 3 M NaAc and 2.5 volume of cold ethanol at –70°C for 1 h, followed by centrifugation at 15,000 rpm for 15 min (4°C). The resulting DNA pellets were washed with 70% ethanol, air-dried, and dissolved in 50 μ l of ddH₂O. Caspase-3 cDNA were obtained by PCR amplification using the two following primers with the sequence specific for caspase-3 underlined: CGCGGATCCGCCACCATGGACTACAAGGACGACGATGACAAGGAGAACTGAAAACACTCAGTG

(5'-primer) and CCGCTCGAGTTAGTGATAAAAAATAGAGTCTTTTTGTGAGC (3'-primer). A *Bam*HI restriction site and a flag tag were added to the 5'-primer and a *Xho*I restriction site to the 3'-primer. Caspase-3s cDNA was amplified using the same 5'-primer as caspase-3 and the following 3'-primer: CCGCTCGAGTCAGCATGGCACAAAGCGACTGGATGAAC. PCR was carried out for 60 s at 95°C, 60 s at 55°C, and 120 s at 72°C for 35 cycles. PCR products were digested with *Bam*HI and *Xho*I endonuclease, gel purified and subcloned into PcDNA 3 vector (Invitrogen).

PCR and Southern hybridization for caspase-3s expression. To examine the expression of caspase-3 variant in normal human tissues and various human cell lines, first-strand cDNA from various human tissues (Multiple Tissue cDNA Panels, Clontech) and from various human cell lines were PCR amplified. The PCR products were resolved in a 1% agarose gel. Gels were soaked in denaturing solution (0.2 M NaOH, 0.6 M NaCl) at room temperature for 30 min, followed by neutralization with buffer (0.5 M Tris-HCl, pH 7.5, 1.5 M NaCl) for 30 min. PCR products were then transferred to a nylon membrane (Life Science Products, Inc.) with 10 \times SSC buffer and were cross-linked by exposure to UV light for 1 min and 45 s. Southern hybridization was performed by first incubating the membrane in prehybridization solution at 60°C for at least 1 h, followed by addition of α -³²P labeled caspase-3 variant cDNA fragment and incubation at 60°C for 12–24 h. The membrane was then washed with washing buffer and exposed to film for 2–24 h at –80°C.

In vitro transcription and translation. *In vitro* transcription and translation were performed using the TNT coupled reticulocyte lysate system (Promega) following the protocol specified by the supplier. Briefly, 0.5–2.0 μ g of caspase-3 and caspase-3s cDNA constructs were translated in the presence of 25 μ l of reticulocyte lysate, 1 μ l of T7 RNA polymerase (Promega), 20 μ M of amino acid mixture minus methionine, and 40 μ Ci of [³⁵S]methionine (Amersham) at 30°C for 2 h. The translated products were resolved in a 4–20% Tris-glycine gel in the presence of SDS running buffer. Gels were then exposed to film at –70°C for 6–18 h.

Transfection and drug treatment. Human embryonic kidney 293 cells were seeded 1 \times 10⁵ into each well of a 24-well plate and transfected with caspase-3 and caspase-3s cDNA constructs by calcium phosphate method (14). Briefly, five hundred nanogram of DNA was incubated in 0.25 M CaCl₂ solution for 10 min at room temperature. The mixture was then incubated with 1 \times Hepes buffer (pH 6.88) for 15 min at room temperature before being added to the culture medium. After 6–7 h transfection, transfection medium was replaced with fresh Opti-MEMI medium (GIBCO) containing 5 μ M of Proteasome Inhibitor I (Z-Ile-Glu(otBu)-Ala-Leu-CHO) (Calbiochem) and incubated for 16 h. For staurosporine treatment, cells were allowed to grow for 24 h in serum containing medium after transfection. Cells then incubated with 0.5 μ M staurosporine (Calbiochem) in Opti-MEMI medium for 20 h.

DNA fragmentation ELISA. DNA fragmentation ELISA was performed using the Cell Death Detection ELISA kit (Boehringer Mannheim) following the instruction provided by the supplier. Briefly, after 16 h treatment with IGAL (Proteasome Inhibitor I), 293 cells were lysed by incubation with 500 μ l of cell lysis buffer for 2 h at room temperature. The lysate were then centrifuged at 200g for 10 min. Twenty microliters of the supernatant were transferred into the streptavidin-coated microtiter plate for analysis using the reagents provided with the assay kit. Statistical analyses were performed with the Student *t* test using Microsoft Excel analysis tools.

Western blotting. Preparation of whole cell lysates and Western blotting were performed as previously described (15, 16). The blots were probed with anti-human caspase-3 p20 goat polyclonal antibody (Santa Cruz, Cat. No. sc-1226), anti-human caspase-3 rabbit polyclonal antibody (Pharmingen, Cat. No. 65906E), anti-flag monoclonal antibody (Sigma, Cat. No. F3165), or anti-human monoclonal

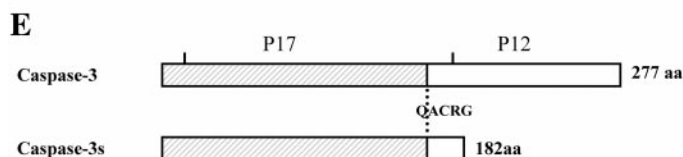
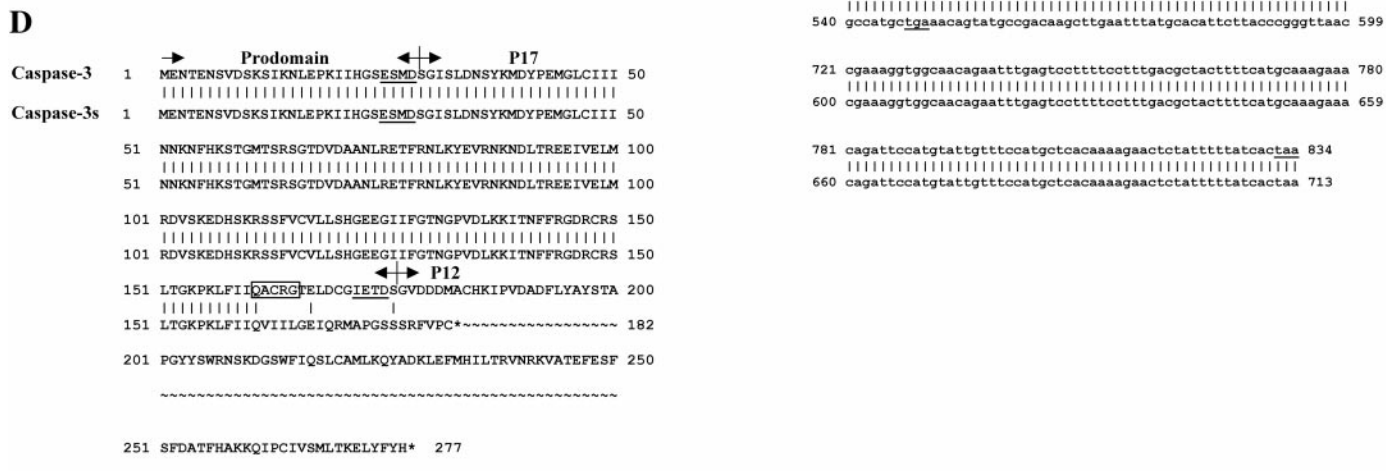
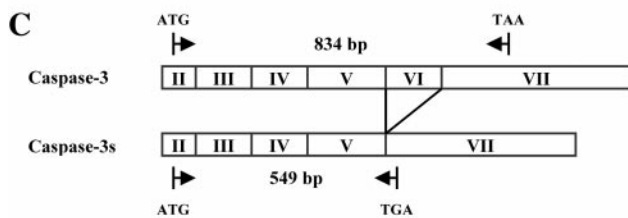
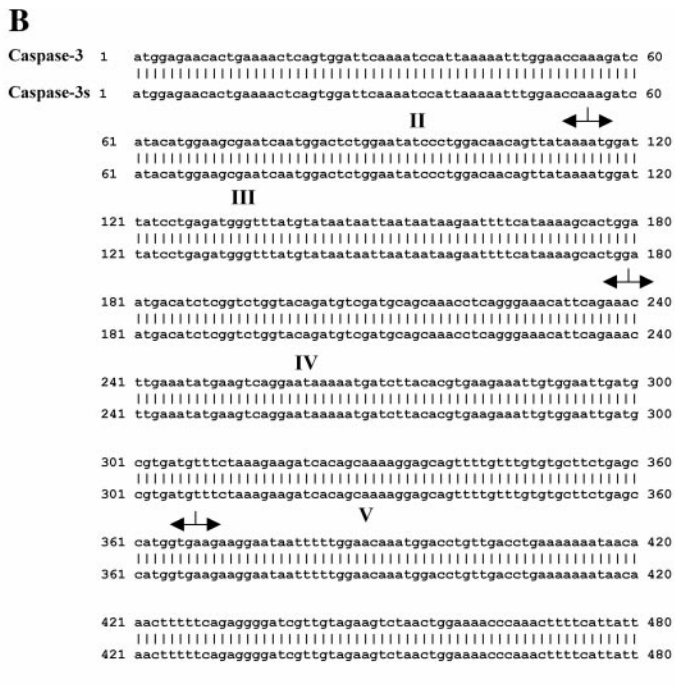
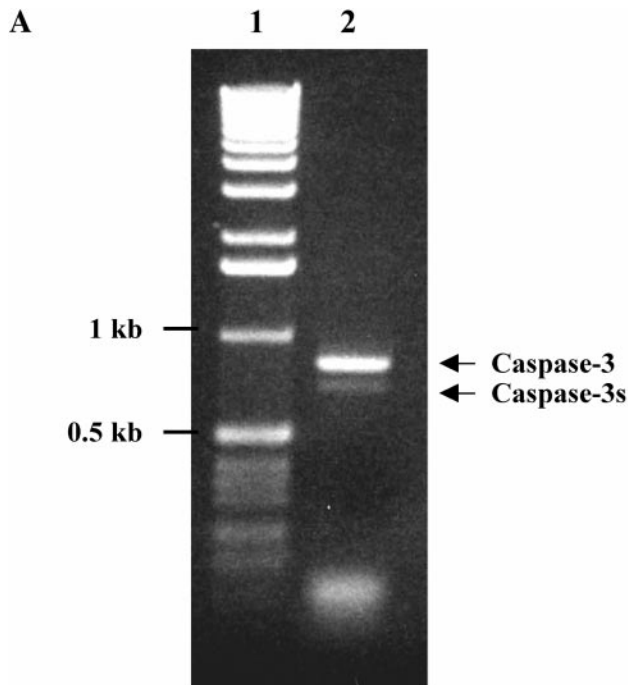


FIG. 1. Caspase-3s is a short variant of caspase-3 that results from alternative mRNA splicing. (A) RT-PCR amplification using primers specific for caspase-3 revealed an additional PCR product of about 700 bp. 1, 1 kb DNA ladder. 2, RT-PCR products amplified from mRNA messages isolated from human colon cancer carcinoma DLD-1 cells. (B) Comparison of the nucleotide sequence of human caspase-3 and human caspase-3s revealed a deletion of exon VI in caspase-3s mRNA. The Roman numerals under the sequences indicate the exons of the caspase-3 gene referred to as the mouse caspase-3 gene (17). The arrows depict the borders of exons. Dotted lines indicate deleted nucleotide

PAPR antibody (Biomol Research Laboratories, Inc. Cat. No. SA-250).

RESULTS

Molecular Cloning of Casapase-3s cDNA

In the process of cloning wildtype caspase 3 by RT-PCR using human carcinoma DLD-1 cell RNA as template, we detected two PCR products with a size of approximately 800 and 700 bp, respectively (Fig. 1A). Nucleotide sequencing revealed that the larger PCR product is the full-length caspase-3 cDNA while the smaller PCR product was its shorter variant (caspase-3s) (Figs. 1B and 1C). Sequence comparison between caspase-3s and caspase-3 revealed a deletion of exon VI according to the genomic organization of the mouse caspase-3 gene (17) (Fig. 1C). This indicated that the caspase-3s was the alternative mRNA splice variant of caspase-3. The deletion of the mRNA sequence resulted in an altered reading frame in the C-terminal, leading to an altered amino acid sequence and a truncated polypeptide (Figs. 1D and 1E). This novel variant is 95 amino acid shorter and shared the same 161 residues with caspase-3 in the N-terminus containing the prodomain and the majority of the large subunit, but its C-terminus lacked the conserved QACRG sequence of caspase-3. The absence of active site cysteine residue in caspase-3s suggested that it might not be a functional protease. To our knowledge, this is the first report of a truncated splice variant of caspase-3.

Coexpression of Caspase-3 and Caspase-3s in Various Normal Human Tissues and Human Cancer Cell Lines

To examine the distribution of caspase-3s expression in normal human tissues, a panel of human multiple tissue cDNA was PCR-amplified using primers specific to full length caspase-3 cDNA. The resulting PCR products were transferred to a nylon membrane and subjected to Southern hybridization using full-length caspase-3s cDNA as probe. The result is shown in Fig. 2A. In all the human tissues examined, caspase-3 and caspase-3s were coexpressed. Although caspase-3 and caspase-3s expression levels vary among different tissues, caspase-3 was usually expressed at a much higher level. We also examined several human cancer cell lines for caspase-3s expression. The mRNA of caspase-3 and caspase-3s were again codetected in three cell lines (293, HeLa, and SH-SY5Y) (Fig. 2B). By

Western blotting of these cell lysates, besides the prominent 32 kDa caspase-3, a 20 kDa species is also detected with an anti-caspase-3 p20 polyclonal antibody. The 20 kDa species is tentatively assigned as caspase-3s, based on its predicted molecular weight. Consistent with other studies, MCF-7 showed no detectable expression of caspase-3 (18), or caspase-3s (Figs. 2B and 2C).

Antiapoptosis Activity of Caspase-3s

To investigate the role of caspase-3s in apoptosis, full-length caspase-3 and caspase-3s cDNA were constructed into pcDNA3 vector. The ability of expression of these constructs was first examined by *in vitro* translation assay as shown in Fig. 3A, caspase-3 and -3s expressed as a 32 kDa and 20 kDa protein, respectively, in a similar level. Surprising, when expression was performed in a transient transfection in 293 cells, caspase-3s was expressed in a much lower level compared to caspase-3 (Fig. 3B).

Since caspase-3s lacks protease active site, it may not have apoptosis-inducing activity and instead might have antiapoptotic properties. To test this hypothesis, 293 cells were transfected with pcDNA3 vector, caspase-3 and caspase-3s cDNA constructs.

The transfected cells were challenged with two apoptosis-inducing agents proteasome inhibitor Z-Ile-Glu(otBu)-Ala-Leu-CHO (IGAL) (19–22) and protein kinase inhibitor staurosporine (23, 24). Overexpression of full-length caspase-3 induced basal apoptosis of 293 cells IGAL as measured by DNA fragmentation ELISA, as expected. Overexpression of caspase-3s partially but significantly inhibited apoptosis induced by IGAL (Fig. 4A). On the other hand, overexpression of caspase-3 did not reduce staurosporine-mediated 293 cell apoptosis.

It is known that cleavage of caspase-3 substrates, such as poly(ADP)ribose polymerase (PARP), is prerequisite for apoptosis. Therefore, we also examined the effects of caspase-3s overexpression on PARP cleavage in both control and IGAL treated 293, along with caspase-3 overexpression as positive control. Cleavage of PARP to an immunoreactive breakdown product (85 kDa) was measured by Western blot. Indeed, caspase-3s overexpression again partially but significantly inhibited the cleavage of PARP compared to the vector control upon treatment with IGAL (lanes 6 vs 4, Figs. 4B and 4C). In contrast, Overexpression of full-length caspase-3 increased basal PARP cleavage in the

sequence compared to caspase-3. The stop codons are underlined and highlighted. (C) Schematic presentation of exon organizations of the caspase-3 and caspase-3s. The region between the two arrows depicts an open reading frame. (D) Amino acid sequence alignment of caspase-3 and caspase-3s. The protein domains are indicated with arrows above the sequence. The cleavage sites for the large and small subunits of caspase 3 are underlined and highlighted. The conserved catalytic sequence is boxed. (E) Schematic presentation of the domain organization of caspase-3 and caspase-3s. The shaded area depicts the amino acid sequence shared by caspase-3 and caspase-3s. The conserved sequence is also indicated in caspase-3, but is missing in caspase-3s.

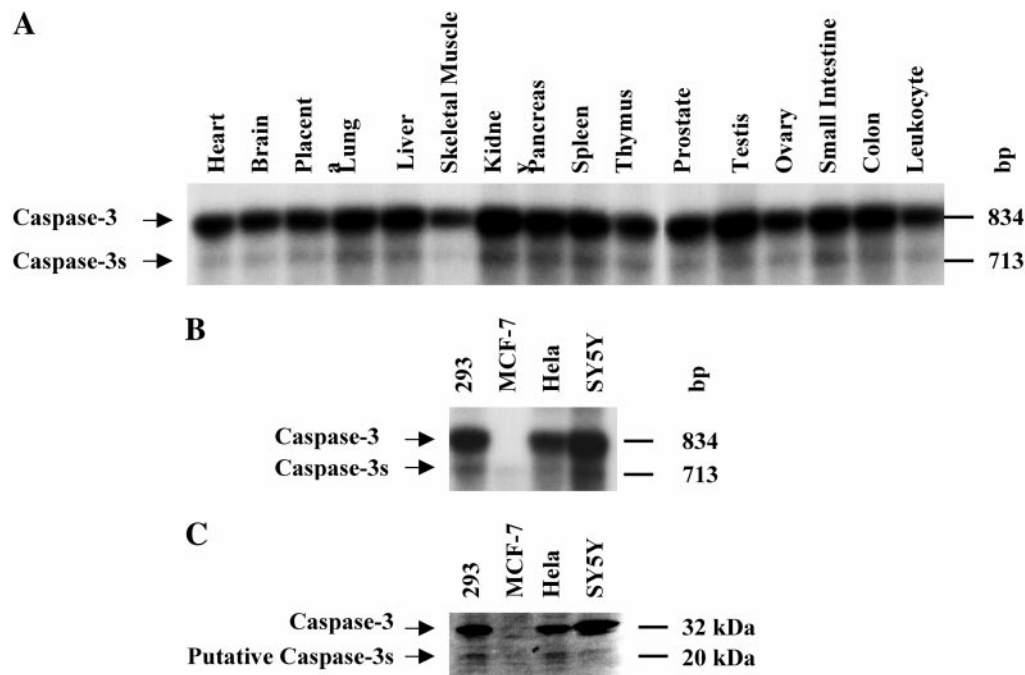


FIG. 2. Coexpression of caspase-3 and caspase-3s in normal human tissues and various human cancer cell lines. (A) PCR and Southern blotting were carried out as described under Materials and Methods. Caspase-3 and caspase-3s were coexpressed in all normal human tissues examined. (B) RT-PCR was carried out with RNA isolated from various cancer cell lines. The PCR products were then subjected to Southern blotting using full-length caspase-3s cDNA as probe. Caspase-3 and caspase-3s were coexpressed in various human cancer cell lines. (C) Western blotting of total cellular protein isolated from various cancer cell lines. The blot was probed with antibody specific for caspase-3 p20. Caspase-3 and a 20 kDa protein were detected in various cell lines, but not MCF-7.

non-treated cells (lane 2), which was enhanced by IGAL treatment (lanes 5 vs 4, Figs. 4B and 4C).

Proteasome Inhibitor Activates Caspase-3 and Stabilizes Caspase-3s

Proteasome appears to regulate caspase-3 potentially in two ways. Proteasome inhibition was reported by many groups to induce caspase-3 processing and activation, via a yet unknown mechanism (21, 22). On the other hand, it was recently shown that full length caspase-3 was ubiquitinated by cIAP2 and potentially directing it toward active degradation by proteasome (25). Since caspase-3s was only expressed in low level in transfected 293 cells, we speculated that it might also be actively and selectively degraded by proteasome. To examine the effects of proteasome inhibitor on the expression of caspase-3 and caspase-3s, total cellular protein from transfected 293 cells treated with proteasome inhibitor IGAL were analyzed by Western blotting probed with the N-terminal anti-flag antibody. Upon IGAL treatment, a decrease in the abundance of recombinant full length caspase-3 (32 kDa band) as well as the appearance of the 20 kDa activated form of caspase-3 (both containing the N-terminal flag-tag) were in fact observed (Fig. 5, lanes 5 vs 2). Therefore, in this cell system, proteasome inhibition-mediated processing and loss of full-length caspase-3 protein ap-

pears to be the predominant pathway. In contrast, we observed a dramatic increase in the amount of recombinant caspase-3s after IGAL treatment in transfected cells (detected by anti-flag-tag antibody) (Fig. 5, lanes 6 vs 3). This indicates that caspase-3s is a rapidly turnover protein, subjected to ubiquitin-proteasome degradation.

DISCUSSION

As one of the characteristics of caspases, all caspases are synthesized as proenzymes, in which the generation of subunits by cleavage and formation of heterotetramers are necessary for enzymatic activities (9, 26). This characteristic ensures the rapid response of cells to various stimuli by a cascade of activation of the corresponding enzymes without the need of novel protein synthesis. However, certain mechanisms are also necessary to protect cells from unwanted suicide and maintain the threshold of response to certain levels of stimuli. One of these mechanisms is the existence of various variants that are encoded from the same gene, but structurally different, usually shorter, due to alternative mRNA splicing. They functionally counteract with their wildtype counterparts. Examples of these caspases include caspase-2 and -9 (27–29). The long forms of these caspases induce apoptosis, whereas

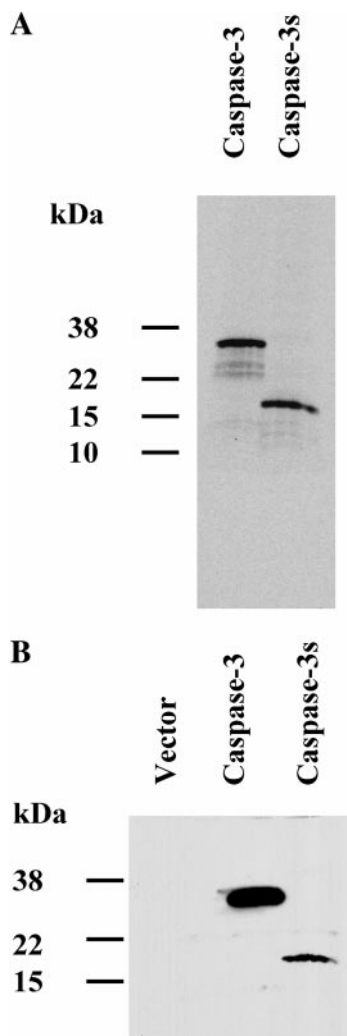


FIG. 3. Expression of caspase-3 and caspase-3s full-length cDNA *in vitro* and in transfected 293 cells. (A) The full-length cDNA fragments encoding caspase-3 and caspase-3s were *in vitro* translated. Translated products were fractionated on a 4–20% Tris–glycine gel and visualized by autoradiography. (B) Caspase-3 and caspase-3s cDNA constructs were transfected into 293 cells. Expression of caspase-3 and caspase-3s were detected by Western blotting using antibody specific for flag-tag.

their short alternative mRNA spliced variants exhibit an antiapoptosis function. The identification of the caspase-3 short variant and its antiapoptosis function in this study has added the importance of such mechanism in programmed cell death.

Eukaryotic cells have two major systems for protein degradation, the lysosomal mechanism and the ATP-dependent cytosolically based mechanism. The later mechanism involves a multi-protein complex, the proteasome. While lysosome degrades proteins nonselectively, proteasome selectively targets proteins that are marked through a process called ubiquitination. The proteasomal protein degradation mechanism selectively eliminates proteins that are damaged and those

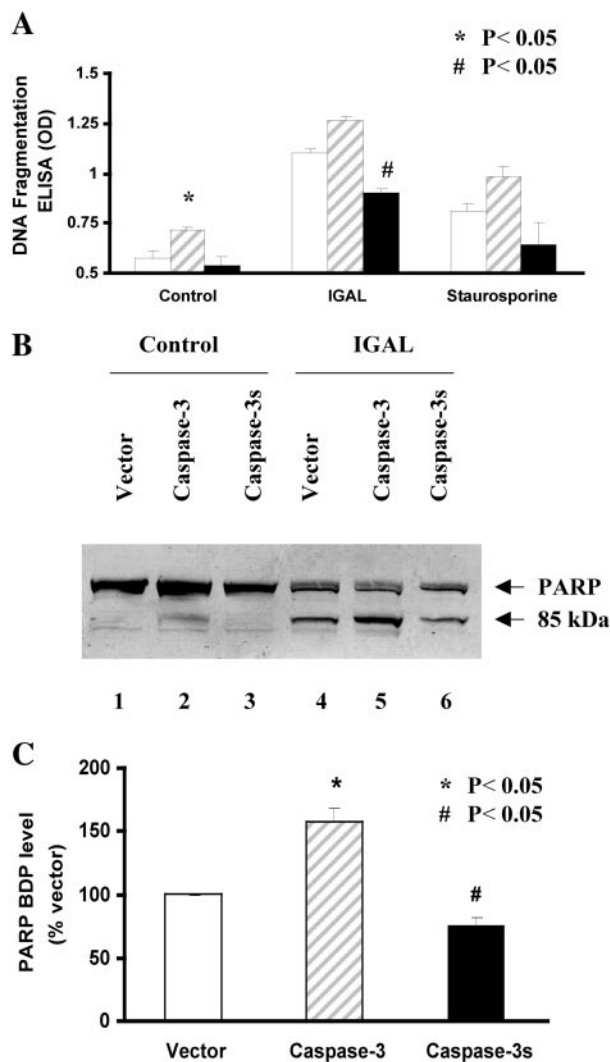


FIG. 4. Overexpression of caspase-3s inhibited apoptosis induced by proteasome inhibitor but not staurosporine. (A) Caspase-3s inhibited cells death induced by proteasome inhibitor I (Z-Ile-Glu(otBu)-Ala-Leu-CHO). pcDNA3 vector, caspase-3, and caspase-3s were transfected into 293 cells. The transfected cells were then treated with 5 μ M of IGAL or 0.5 μ M staurosporine. DNA fragmentation was measured by ELISA after 16–20 h of treatment. In IGAL-treated cells, transfection of caspase-3s significantly ($\#P < 0.05$) inhibited DNA fragmentation compared to the vector control and caspase-3-transfected cells after the treatment ($n = 4$). For untreated cells, transfection of caspase-3 significantly increased DNA fragmentation compared to both vector and caspase-3s-transfected cells ($*P < 0.05$). Caspase-3s had no significant effect on apoptosis induced by staurosporine treatment. (B) Caspase-3s partially inhibited PARP cleavage after treatment with proteasome inhibitor compared to the vector control. 293 cells were transfected with caspase-3, caspase-3s or pcDNA3 vector followed by IGAL treatment for 16 h or left untreated (control). PARP expression is measured by Western blot. (C) Densitometric quantification of PARP breakdown product (85 kDa) from B ($n = 8$). The results were normalized to vector control and presented as percentage. The PARP breakdown product in caspase-3s-transfected cells was significantly decreased while transfection of caspase-3 increased PARP cleavage. Both were compared to the vector control.

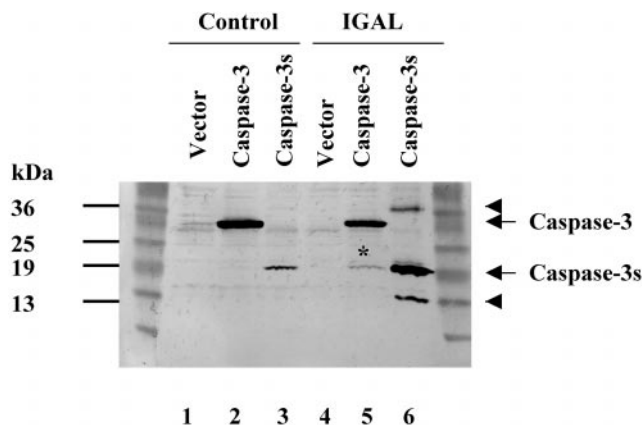


FIG. 5. Proteasome inhibitor activated caspase-3 and stabilized caspase-3s. (A) 293 cells were transfected with flag-tagged caspase-3, flag-tagged caspase-3s or pcDNA3 vector, followed by IGAL treatment for 16 h or left untreated (control). Fifty micrograms of total cellular protein was assayed for caspase-3 and caspase-3s expression using antibody specific for flag-tag. The asterisk (*) indicates the N-terminal flag-tag containing activated caspase-3 (20 kDa). The uppermost and lowest short arrow denote the two additional bands, 40 and 14 kDa, respectively. (B) Proteasome inhibitor treatment stabilized endogenous caspase-3 expression. 293 cells were treated with 5 μ M IGAL for 48 h. Total cell lysates were isolated and assayed for caspase-3 expression by Western blotting. The blot was probed with anti-caspase-3 antibody (Pharmingen).

that interfere with normal cellular processes. This mechanism is therefore critical for cell survival. A growing body of literature has revealed the involvement of such mechanism in programmed cell death. Inhibition of proteasome function has been shown to activate caspases and induce apoptosis (19–22). Consistent with these studies, our results showed an induction of apoptosis and activation of caspase-3 by inhibition of proteasome function (Figs. 4 and 5). Furthermore, our results also revealed an additional role of proteasome in the degradation of caspase-3s. This conclusion is based upon the observation that (i) caspase-3s were stabilized by proteasome inhibitor IGAL (Fig. 5) and (ii) apoptosis induced by proteasome inhibition, but not by staurosporine, was significantly suppressed by caspase-3s overexpression (Fig. 4). Taken together, these data suggest that proteasome inhibition has at least two opposing effects: (i) induction of apoptosis by activating caspase-3 (via a still unknown mechanism) and (ii) antiapoptosis effect by stabilizing caspase-3s. Since endogenous caspase-3s is generally expressed in a much lower level compared to caspase-3 (Figs. 2A, 2B, and 2C), despite its stabilization by proteasome inhibitor, the caspase-3s level is not sufficient to completely suppress apoptosis. However, it is conceivable that under certain chronic pathophysiological conditions (e.g., carcinogenesis and neurodegenerative diseases), an alternation of proteasome accessibility could lead to an imbalance of the caspase-3s/caspase-3 ratio and thus alter the cells' susceptibility

to apoptosis. Therefore, the proteasome pathway might play a complex and dynamic role in both healthy cells and cells undergoing apoptosis (30). This is a subject that requires further study.

In summary, we cloned and characterized a caspase-3 short variant that is resulted from alternative mRNA splicing. This caspase-3 short variant is naturally coexpressed with wildtype caspase-3 and has antiapoptosis properties. Since caspase-3 has such a critical role in both physiologic and unscheduled apoptosis, our findings are potentially important in terms of development of carcinogenesis and/or the neurodegenerative diseases, where altered expression of this caspase-3 variant may lead to the change of cell death rate.

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