Communication

CPP32, a Novel Human Apoptotic Protein with Homology to Caenorhabditis elegans Cell Death Protein Ced-3 and Mammalian Interleukin-1β-converting Enzyme* (Received for publication, September 30, 1994)

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We have cloned a novel apoptotic gene from human Jurkat T-lymphocytes. The new gene encodes a 32-kDa putative cysteine protease (CPP32) with significant homology to Caenorhabditis elegans cell death protein Ced-3, mammalian interleukin-1β-converting enzyme (ICE), and the product of the mouse nedd2 gene. The CPP32 transcript is highly expressed and most abundant in cell lines of lymphocytic origin. Overexpression of CPP32 or ICE in Sf9 insect cells resulted in apoptosis. In addition, coexpression of recombinant p20 and p11 derived from the parental full-length CPP32 sequence resulted in apoptosis in Sf9 cells. Our data suggest that similar to ICE, CPP32 is made of two subunits, p20 and p11, which form the active CPP32 complex. The apoptotic activity of CPP32 and its high expression in lymphocytic cells suggest that CPP32 is an important mediator of apoptosis in the immune system.

Apoptosis is a selective, controlled, and genetically programmed cell death process, which occurs as a result of normal cellular differentiation and development of multicellular organisms (1–3). It is also involved in tissue homeostasis, aging, and pathological processes (1–3). In recent years, a new class of gene products was identified as inducers of apoptosis in a variety of organisms. These gene products include mammalian ICE and Ned2 proteins and Caenorhabditis elegans Ced-3 (4–6). Interestingly, all three proteins share significant sequence and structural homology (5, 6). ICE and its homologs are classified as a new class of cysteine proteases because they are different in sequence, structure, and substrate specificity from other known cysteine proteases (7–9). All three proteins have been shown to induce apoptosis when overexpressed in different cell types (4, 5). This apoptosis was inhibited by coexpression of the antiapoptotic protein Bcl2 (4, 5). Expression of crmA, a poxvirus-specific inhibitor of ICE (10), inhibited ICE-induced apoptosis in fibroblasts (4). crmA also inhibited apoptosis of ganglion neuronal cells when introduced into these cells (11). Because crmA could inhibit other ICE homologs, it is not yet clear whether ICE or other members of this new class of cysteine proteases are involved in neuronal apoptosis.

Because of the importance of ICE-related cysteine proteases in apoptosis, we were interested in identifying other members of this important class of cysteine proteases. In this report we describe the cloning, expression, and partial characterization of a novel putative cysteine protease called CPP32. We show that CPP32 is related to Ced-3, ICE, and Nedd2 proteins and can cause apoptosis when expressed in Sf9 insect cells.

MATERIALS AND METHODS

Cloning of CPP32—Searching the GenBank expressed sequence tags (12) for sequences with homology to Ced-3 or ICE, we identified a human sequence of 399 bp (GenBank accession number T10341) with significant homology to Ced-3 and ICE. This sequence was then cloned by a combination of reverse transcription and polymerase chain reaction techniques (RT-PCR). Reverse transcription was performed on poly(A)+ RNA from the human T-lymphocyte cell line Jurkat using a 17-mer poly(T) primer and Moloney murine leukemia virus reverse transcriptase. The reverse transcription product was then used as a template for PCR using two specific primers, hecd1 and hecd2, derived from the GenBank T10341 sequence. Primer sequences were as follows: hecd1, CAGAGGGATCG'GTAGAAG hced2, GTTGCCACCTTTCG-30761

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U13737 and U13738.

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The amplified DNA was blunt-ended with T4 DNA polymerase, phosphorylated with T4 polynucleotide kinase, and cloned into a Smal-cut pBluescript II KS+ vector (Stratagene). The cloned cDNA was sequenced with T3 and T7 sequencing primers and found to match exactly the T10341 cDNA. This cDNA was then excised from the vector, radiolabeled, and used to screen a Jurkat λ Uni-ZAP9 XR CDNA library constructed in our laboratory. Twenty λ clones were selected, purified, and then rescued from the λ Zap phage clones into the pBluescript II SK+ plasmid vector. The plasmid clones were characterized by restriction enzyme analysis and nucleotide sequencing.

Construction of Plasmids, Transfer Vectors, and Recombinant Baculoviruses—The CPP32 cDNA was excised from the pBluescript II SK+ vector as a 1-kilobase cDNA fragment with EcoRI and PstI restriction enzymes and subcloned into an EcoRI/PstI-cut pVL1393 to generate the recombinant transfer vector pVL-CPP32. The cDNA fragment contains 57 bp of untranslated 5' sequence, the entire open reading frame, and 107 bp of untranslated 3' sequence. The cDNAs for the p20 and p11 subunits of CPP32 were generated by PCR using synthetic primers (p20ATG and p20TGA for p20; p11ATG and P11TAA for p11) and pVLP-CPP32 as a template. Primer sequences were as follows: p20ATG, ATGAGGAAACAGTAAAACACG; p20TGA, GTGCATCATACAACCTAGCT; p11ATG, ATGGCGTGTCATAAAATACCAG; p11TAA, CCAAACACATATTTGTAGT.

The amplified DNA fragments were blunt-ended, phosphorylated, and then cloned in the Smal site of pVL1393 transfer vector under the polyhedrin promoter and designated as pVL-p20 and pVL-p11. Human ICE full-length cDNA was obtained by RT-PCR from Jurkat T lymphocyte RNA and cloned into the BamHI/EcoRI sites of pVL1393.2 All recombinant transfer vectors were then used to generate recombinant baculoviruses as described previously (13, 14).

3 E. S. Alnemri, T. Fernandes-Alnemri, and G. Litwack, submitted for publication.
Cloning of Human CPP32

RESULTS AND DISCUSSION

Cloning of CPP32—One way to identify mammalian genes involved in apoptosis is to search for and isolate genes with homology to C. elegans ced-3 gene or mammalian ICE gene. Using the DNA sequence encoding the active site region of mammalian ICE or C. elegans Ced-3 to search the Uni-ZAP™ cDNA library, this resulted in the isolation of several cDNA clones. The sequence of one of these clones is shown in Fig. 1. This cDNA, termed isoform-α, contains an open reading frame of 2652 nucleotides with a predicted molecular mass of ~32 kDa. The initiator methionine at nucleotide 225 conforms to the consensus Kozak translation initiation sequence (15) and is preceded by an in-frame nonsense codon (TAG) at position 135. A second cDNA clone was also sequenced and found to contain a deletion of 30762 bps including the first 14 bp that contains the start codon. This deletion is probably due to alternative splicing.

CPP32 lacks the long N terminus, which is most probably not required for activity. The highest degree of homology between the four proteins lies within and around the region that contains the highly conserved pentapeptide QACRG (Fig. 2A). This pentapeptide contains the active site cysteine residue (Cys-28) of ICE and could have a similar function.

ICE is classified as a cysteine protease (7). The active form of ICE is generated after proteolytic cleavage of the proenzyme p45 to generate two subunits with molecular mass values of 20 and 10 kDa (Fig. 2B). Two p20/p10 heterodimers associate with each other to form the active ICE tetrameric complex (8, 9). Structural analysis of CPP32 based on its homology to ICE revealed that Asp-175 and Asp-341, which are involved in substrate binding and catalysis (8, 9), are conserved in all four proteins. The side chains of ICE residues Arg-179 and Arg-341 recognize the aspartate at P1 position of the substrate by direct charge-charge interaction (8, 9). Based on these observations CPP32 could have the same substrate requirement as ICE, that is, it requires Asp in the P1 position. Whether CPP32 can cleave interleukin-1β remains to be established.

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Novel Putative Cysteine Protease—A search of the SWISS-PROT data base revealed that the predicted CPP32 protein sequence is similar to the C. elegans Ced-3 protein (6), mammalian ICE, and mouse Nedd2 protein (5, 7, 16) (Fig. 2A). CPP32 is most similar to Ced-3: CPP32 shows 35% identity (58% similarity) with Ced-3, 30% identity (53% similarity) with human ICE, and 30% identity (53% similarity) with mouse Nedd2 protein. Ced-3, ICE, or Nedd2 are less than 31% identical with each other. This suggests that CPP32 is more related to Ced-3 than to ICE or Nedd2 and could have a similar function to Ced-3. However, unlike Ced-3, ICE, or Nedd2, CPP32 lacks the long N terminus, which is most probably not required for activity. The highest degree of homology between the four proteins lies within and around the region that contains the highly conserved pentapeptide QACRG (Fig. 2A). This pentapeptide contains the active site cysteine residue (Cys-28) of ICE and could have a similar function.

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expression of human CPP32 with other members of the cysteine protease family. A, collinear sequence alignment of human CPP32 with C. elegans CED-3, human ICE, and mouse Nedd2 protein. Dotted lines indicate gaps in the sequence to allow optimal alignment. Amino acid residues are numbered to the right of each sequence. Amino acids identical in at least three out of four sequences are boxed. The conserved pentapeptide containing the active site Cys in ICE is boxed and shaded. Asterisks indicate known aspartate cleavage sites between the two subunits of ICE which are conserved in the other sequences. B, structural comparison of CPP32 and ICE. The ICE and CPP32 proenzymes are represented as rectangles. The four known aspartate cleavage sites in ICE and the two potential aspartate cleavage sites in CPP32 are indicated. The two subunits of active ICE (p20 and p10) and the two putative subunits of active CPP32 (p20 and p11) are indicated by solid arrows.

Fig. 2. Sequence and structural comparison of the human CPP32 with other members of cysteine protease family. A, collinear sequence alignment of human CPP32 with C. elegans CED-3, human ICE, and mouse Nedd2 protein. Dotted lines indicate gaps in the sequence to allow optimal alignment. Amino acid residues are numbered to the right of each sequence. Amino acids identical in at least three out of four sequences are boxed. The conserved pentapeptide containing the active site Cys in ICE is boxed and shaded. Asterisks indicate known aspartate cleavage sites between the two subunits of ICE which are conserved in the other sequences. B, structural comparison of CPP32 and ICE. The ICE and CPP32 proenzymes are represented as rectangles. The four known aspartate cleavage sites in ICE and the two potential aspartate cleavage sites in CPP32 are indicated. The two subunits of active ICE (p20 and p10) and the two putative subunits of active CPP32 (p20 and p11) are indicated by solid arrows.

Expression of CPP32 Transcript in Different Human Cell Lines—We examined the potential tissue distribution of CPP32 mRNA using different human tumor cell lines. As shown in Fig. 3, CPP32 mRNA is detectable in all cell lines examined. Interestingly, CPP32 mRNA was highly expressed in cell lines of hematopoietic lineage such as lymphocytes and promyelocytes (Fig. 3, lanes 1-9). High expression was also observed in cell lines of brain and embryonic origins such as A173 (lane 13) and 293 (lane 15), respectively. In contrast, ICE mRNA was not detectable by Northern blot analysis in any of the lymphocyte cell lines described in Fig. 3. ICE mRNA was only detectable by RT-PCR in those cell lines (data not shown). A previous study has shown that peripheral blood T- and B-lymphocytes express substantially small amounts of ICE mRNA compared with peripheral blood monocytes and neutrophils (16). The high level of expression of CPP32 in cells of the immune system suggests that CPP32 could play an important role in regulation of apoptosis in the immune system. The immune system is one in which apoptosis occurs most frequently in response to many different stimuli.

Expression of CPP32 in Sf9 Cells Induces Apoptosis—The baculovirus gene product p35 is an antiapoptotic protein that protects insect cells against baculovirus-induced apoptosis (17). Sf9 cells infected with the wild type baculovirus AcNPV remain viable 48-72 h post-infection due to the protective effect of p35.

Fig. 2. Northern blot analysis of the expression of human CPP32. Total cellular RNA was isolated from the following human tumor cell lines: Peer (lane 1), SupT1 (lane 2), CEM C7 (lane 3), CEM C1 (lane 4), Molt4 (lane 5), and Jurkat (lane 6) T-lymphocytes; 697 (lane 7) and 380 (lane 8) pre-B lymphocytes; K562 (lane 9), a promyelocyte; HeLa (lane 10), a cervical carcinoma; A431 (lane 11), a vulva carcinoma; Colo20 (lane 12), a colon adenocarcinoma; MCF7 (lane 13), a breast carcinoma; A173 (lane 14), a glialbloma; 293 (lane 15), an Ad-5-transformed embryonic kidney fibroblast. Lane 16 contains poly(A) mRNA from Jurkat T-lymphocytes. Equal amounts of the RNA samples (15 µg/sample) were fractionated on formaldehyde-agarose gel as described under "Materials and Methods." The upper panel shows CPP32 mRNA detected with a CPP32-specific probe. The lower panel shows the same blot stripped and reprobed with a human FKBP52-specific probe as a control. FKBP52 is expressed in most cells. The positions of the 18 and 28 S RNAs are indicated on the left.
obtained with recombinant p10 and p20 subunits of ICE. We also tested whether a heterodimer can be formed by coexpression of pICE-p20 and CPP32-p11 or vice versa (i.e., CPP32-p20 and ICE-p10), and whether they could induce apoptosis in Sf9 cells. These combinations were unable to cause apoptosis in Sf9 cells (data not shown). These observations suggest that a p20 subunit derived from ICE is unable to heterodimerize with the p11 from CPP32 and vice versa. Another possibility is that ICE-p20/ CPP32-p11 or CPP32-p20/ICE-p10 heterodimers are inactive.

In conclusion, we have cloned a new member of the cysteine protease family. The structure of this new protein is similar to that of ICE, in that it is made up of two subunits derived from one precursor proenzyme. Similar to ICE and the other members of this family such as Ced-3 and Dedd2, this protein could be a mediator of apoptosis in human tissues, especially in cells of the immune system, where we have found it to be highly expressed. The cloning and characterization of CPP32 will aid the efforts to understand the function and regulation of this class of cysteine proteases and their participation in the molecular mechanism of apoptosis.

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