Identification and Characterization of a Pro-tumor Necrosis Factor-α-processing Enzyme from the ADAM Family of Zinc Metalloproteases

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Tumor necrosis factor-α (TNF) is initially expressed as a 26-kDa membrane-bound precursor protein (pro-TNF) that is shed proteolytically from the cell surface, releasing soluble 17-kDa TNF. We have identified human ADAM 10 (HuAD10) from THP-1 membrane extracts as a metalloprotease that specifically clips a peptide substrate spanning the authentic cleavage site between Ala76 and Val77 in pro-TNF. To confirm that HuAD10 has ADAM 10 (HuAD10) from THP-1 membrane extracts as a converting enzyme, we cloned, expressed, and purified an active, truncated form of HuAD10. Characterization of recombinant HuAD10 (rHuAD10) suggests that this enzyme has many of the properties (i.e. substrate specificity, metalloprotease activity, cellular location) expected for a physiologically relevant TNF-processing enzyme.

 MATERIALS AND METHODS

Reagents—Dinitrophenol-labeled polypeptides were synthesized by the Fmoc (N-(9-fluorenlymethyl)oxycarbonyl)-butyl-based solid phase peptide chemistry method using an Applied Biosystems, Inc. 431A peptide synthesizer (10). All peptides were purified by reversed phase HPLC, and molecular weights were verified by mass spectrometry. HPLC Peptide Assay—TNF processing activity was measured as the ability to cleave a 12-residue peptide spanning the Ala76-Val77 site in pro-TNF. The chromatographic peptide substrate DNP-SPLAQA-VRSSR-COHNH2 was dissolved to a final concentration of 0.5 mg/ml in 40 mM Tris-HCl, 100 mM ZnCl2, 10% glycerol, 0.5% octylglucoside. Protein samples were diluted 1:1 with the peptide substrate and allowed to incubate for 1 h or longer at 37 °C, depending on the relative activity of the sample. The unhydrolyzed substrate and cleaved peptides were separated using a Beckman System Gold HPLC with a Vydac C18 reversed phase column (4 mm × 30 cm), and the eluate was monitored at 350 nm. The peptides were eluted using linear gradients of CH3CN/0.1% trifluoroacetic acid. Mass spectrometry confirmed that the peptide fragments produced by the partially purified protein or rHuAD10 had the correct molecular weight. One milligram of enzyme activity is defined as 1 nmol of peptide substrate cleaved/min/mg of protein at 37 °C.

Cells and Culture Conditions—THP-1 cells (American Type Culture Collection, Rockville, MD) were grown in RPMI (Life Technologies, Inc.), 1% glutamine penicillin-streptomycin (Life Technologies, Inc.), and 10% heat-inactivated fetal bovine serum.

Membrane Extraction—THP-1 membranes were prepared essentially by the method of Maeda et al. (11) except that dithiothreitol was omitted, and 200 μM phosphoramidon was added to all of the buffers. Membranes were homogenized in buffer containing 10 mM Tris-HCl, pH 8.0, 10% glycerol, 1 mM PefablocSCM, 200 μM phosphoramidon, 1.3 mM aprotinin, 5 μM leupeptin, 30 mM NaCl. Ocythioligocoulate and NaCl were next added to final concentrations of 2% and 0.5 M, respectively. The mixture was rocked at 4 °C for 3 h and then centrifuged at 180,000 g x g for 1 h. The supernatant was recovered and assayed for TNF processing activity.

S-Sepharose (HP) Column Chromatography—Membrane extracts were diluted 25-fold with buffer A (20 mM NaOAc, 20 mM monobasic sodium phosphate, pH 5.0, 10% glycerol, 0.5% octylglucoside, 50 μM phosphoramidon) and brought to a NaCl concentration of 20 mM. The mixture was clarified by filtration before being loaded onto an S-Sepha-
rose column previously equilibrated with buffer A. The proteins were eluted in 10 column volumes with a linear gradient to 100% of buffer B (20 mM NaOAc, 20 mM dibasic sodium phosphate, pH 10.0, 0.5% glycerol, 0.5% octylglucoside, 50 μM phosphoramidon). The pH of the collected fractions was adjusted immediately to 8.0 with 1 M Tris base.

### Wheat Germ Lectin Affinity Chromatography

The active S-Sepharose fractions were pooled and diluted 1:1 with buffer C (30 mM Tris-HCl, pH 7.0, 10% glycerol, 150 mM NaCl). Additional protease inhibitors were added to final concentrations of 1 μg/ml pepstatin, 1 mM PefablocS™, 2 μg/ml aprotinin, and 50 μM phosphoramidon. The protein pool was next applied to a wheat germ lectin-Sepharose column equilibrated in buffer C. The column was washed with additional buffer C and eluted with 500 mM N-acetylglucosamine. A majority of the original activity was present in the flow-through (69%) which was taken forward onto the next column.

### Hydroxyapatite (HA) Chromatography

The wheat germ flow-through pool was concentrated, diluted 1:4 with buffer D (10 mM sodium phosphate, pH 7.6, 10% glycerol, 0.5% octylglucoside, 50 μM phosphoramidon), loaded onto an HA-Ultralight column, and eluted with a linear gradient from 10 to 400 mM sodium phosphate in buffer D. A majority of the activity was detected in the flow-through, whereas many of the contaminating proteins bound to the HA resin.

### Protein Sequencing

The HA flow-through was concentrated and the components separated by SDS-PAGE using a 10% Tris-glycine gel and eluted in buffer D (10 mM sodium phosphate, pH 7.0, 10% glycerol, 150 mM NaCl). Additional protease inhibitors were added to final concentrations of 1 μg/ml aprotinin, and 50 μM phosphoramidon, before loading onto a butyl-Sepharose HP column equilibrated in buffer D. The wheat germ flow-through pool was concentrated, diluted 1:4 with buffer D (10 mM sodium phosphate, pH 7.6, 10% glycerol, 0.5% octylglucoside, 50 μM phosphoramidon), loaded onto an HA-Ultralight column, and eluted with a linear gradient from 10 to 400 mM sodium phosphate in buffer D. A majority of the activity was detected in the flow-through, whereas many of the contaminating proteins bound to the HA resin.

### Expression of rHuAD10—HuAD10 cDNA lacking the transmembrane domain and cytoplasmic tail coding sequences was fused in-frame to a human IgG1 heavy chain hinge and CH2 and CH3 domains. The HuAD10 portion was prepared by PCR amplification of a 2,038-bp DNA fragment spanning the initiation codon to the codon corresponding to Glu672. The 5'-DNA oligonucleotide primer (5'-ACT TAT TAA GAT GTC CCA AGC AGA TGG TGT TCG (CT)GTA (CG)AG TG) derived from the 5'-ends of bovine (12) and murine sequences and a primer (5'-AAG CTA ATT GCG GCC GGC AAA AGG TGT AGT TAG CTG ATG) derived from the 3'-end of the human sequence (12). The PCR was carried out using Advantage polymerase as described by the manufacturer (Clontech, Palo Alto, CA), with 2 × 10² plaque-forming units of a human macrophage cDNA library cloned in AZAPIT™ (Stratagene, La Jolla) as template. A single band of the expected size, 2.2 kilobases, was generated using these conditions. The PCR was then purified in the presence of radiolabeled nucleotide, and the [32P]-labeled product was used to probe the human macrophage cDNA library used in the PCR.

Positive hybridizing clones were plaque-purified, and phagemid DNA (pHuAD10) was excised as described by the manufacturer (Stratagene).

For mammalian expression, 2 × 10⁶ 293-EBNA cells (Invitrogen) were transfected with 10⁷ plaque-forming units of a human immune system multiple tissue (2) and murine sequences and a primer (5'-GTG GCA CCA AGC TTG CCA CCA TTG TGT TCG TGT TCG TAA TCT) contained a HindIII restriction site and a Kozak sequence, whereas the 3'-oligonucleotide primer (5'-GAG CGC GCG GGC TGC AAT GTT TTT ATG AAG CTC) contained a NotI restriction enzyme site. PCR was performed on pHuAD10 using Pfu polymerase as described by the manufacturer (Stratagene). The resulting PCR product was then digested with HindIII and NotI and then ligated into the human IgG vector, pFC-A3. The HindIII-XhoI fragment containing HuAD10-Ig was then cloned into the mammalian expression vector, pcPE4 (Invitrogen, San Diego) to create pHuAD10-Ig.

For mammalian expression, 2 × 10⁶ 293-EBNA cells (Invitrogen) were transfected with 10 μg of pHuAD10-Ig using LipofectAMINE™ as described by the manufacturer (Life Technologies, Inc.). After 24 h, the cells were split 1:5 into medium containing 100 μM hygromycin. The drug-resistant cells were expanded and used to seed Nunc cell factories at a density of 1.4 × 10⁶ cells/ml. The cells were washed with phosphate-buffered saline and then incubated in serum-free Dulbecco's modified Eagle's medium containing 1 % r-glutamine and 100 μg/ml hygromycin. The medium was conditioned for 7 d at 37 °C before harvesting.

### Purification of rHuAD10—6 liters of conditioned medium from THP-1 cells was concentrated 10 × using a Filtron 50-kDa filter and dialyzed extensively against 10 mM sodium phosphate, pH 7.0, 10% glycerol (buffer E). The active HA fractions were pooled and dialyzed against 50 mM sodium phosphate, 1.5 mM ammonium sulfate, pH 7.0 (buffer F) before being loaded onto a butyl-Sepharose HP column equilibrated in buffer F. rHuAD10 was recovered from the resin using a inverse salt gradient from 1.5 M to 0.5 M ammonium sulfate in 50 mM sodium phosphate, pH 7.0. Active fractions from the butyl-Sepharose column were concentrated, buffer exchanged in 150 mM ammonium bicarbonate, 0.3% CHAPS, pH 8.1, then applied to a Superdex 200 (HR 10/30) gel filtration column.

### HuAD10 Immunoblotting

A peptide (DANQPEGRKKLKLPGK) derived from amino acids 486–502 in rHuAD10 was synthesized and injected into rabbits to generate polyclonal antiserum AMG1961. For immunoblotting, samples were subjected to SDS-PAGE, and proteins were transferred electrophoretically to nitrocellulose. Filters were incubated in blocking buffer (5% skim milk in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) for 1 h at room temperature and then treated with AMG1961 (1:1,000) in blocking buffer for 2 h at room temperature. After washing, filters were incubated with anti-rabbit IgG (Fe) AP conjugate (Promega, Madison, WI) in blocking buffer for 1 h at room temperature. Immune complexes were visualized by incubating with Western Blue stabilized substrate for alkaline phosphatase (Promega) according to the manufacturer's specifications.

### In Vitro Translation and Cleavage of Pro-TNF

The human pro-TNF coding sequence was inserted into the EcoRI site of pCRII (Invitrogen). Plasmid DNA was transcribed and translated using a rabbit reticulocyte lysis translation kit (TNT™ SP6-coupled Reticulocyte Lysate System, Promega) with [35S]cysteine (Amersham Corp.), according to the manufacturer's recommendations. The lysate was dialyzed against a 250-fold excess volume of buffer consisting of 50 mM Tris-HCl, pH 8.5, 10% glycerol, and 10 mM NaCl for 3 h at 4 °C. After dialysis, pro-TNF
was incubated with a partially purified protein preparation with TNF processing activity (S-Sepharose pool) or rHuAD10 50 mM ZnCl₂ for 10 h at 37 °C. The reaction mixtures were immunoprecipitated with rabbit anti-human TNF polyclonal antibody (Amgen Inc.) before SDS-PAGE analysis.

RESULTS

Purification of TNF Processing Activity—

A purification scheme for the isolation of a TNF-processing enzyme from THP-1 membrane extracts was optimized using an assay based on the specific proteolysis of a peptide spanning the pro-TNF cleavage site. Purification recoveries after cation exchange, lectin affinity, and hydroxyapatite chromatographies are shown in Table I. During the final column step, we saw a decrease in specific activity which was indicative of protein instability, even in the presence of detergents and glycerol. A protein blot from a final preparation with TNF processing activity is shown in Fig. 2. Nucleotide and deduced amino acid sequence for HuAD10. The putative signal sequence, transmembrane sequence, and propeptide cleavage site are indicated by lowercase letters, underlining, and a solid arrow, respectively. Light shading is indicative of the metalloprotease active site, and dark shading is indicative of the disintegrin loop. Four putative N-linked glycosylation sites are circled, and two potential SH3 (Src homology 3) domains are boxed.
activity is shown in Fig. 1. Although we were unable to purify the active component to homogeneity, the correct proteolytic activity consistently tracked with the presence of a 62-kDa protein having an NH₂ terminus of SAEKNTXQLYQTDDHLFFKYTYG. A search of this sequence against GenBank revealed a high homology with the NH₂ terminus of BoAD10. This protease is the 10th member of a family of proteins known to contain both "a disintegrin and metalloprotease domain." ADAM 10 was previously purified from bovine brain, but its physiological function was not known (12). Copurifying with HuAD10 were nonproteolytically active proteins including bovine serum albumin (from the growth medium), I-plastin (an actin-binding protein), and β-N-acetylated hexosaminidase α-chain.

Cloning of HuAD10 cDNA—The initial identification of HuAD10 in an active, highly purified THP-1 cell extract prompted us to clone and express rHuAD10 for further characterization studies. A search of GenBank revealed that a partial HuAD10 cDNA lacking the 5'-end had been deposited by P. Glynn (12). PCR and hybridization techniques were used to identify a full-length cDNA from a human macrophage library.

The HuAD10 cDNA is 3,410 bp (Fig. 2) consisting of a 469-bp 5'-untranslated region, a 2,244-bp sequence encoding a protein of 748 amino acids, and a 697-bp 3'-untranslated region. Northern blot analysis (Fig. 3A) demonstrated that a single transcript of approximately 5.0 kilobases (also detected by Howard et al. (12) in five human cell lines) was present in all tissues examined. When the same blot was hybridized with a TNF-specific probe, TNF mRNA was detected in three of the six tissues tested (Fig. 3B). The deduced amino acid sequence of our clone is identical to the published partial sequence of HuAD10 except that we observed a glycine at amino acid 296 instead of a serine. Overall, HuAD10 is 96.7% identical to BoAD10 and 97.1% identical to a partial cDNA encoding rat ADAM 10 (12).

Comparisons with other family members indicate that HuAD10 is most closely related to TACE (49.8% overall similarity) (7, 8) followed by murine ADAM 8, a macrophage surface antigen (49.4% overall similarity) (13)). Analogous to other ADAM family members, HuAD10 possesses propeptide, metalloprotease, disintegrin, and transmembrane domains (Fig. 2) (14). The HuAD10 propeptide is predicted to end at amino acid 213 with a tetradecin motif (RKKR) that might serve as a cleavage site for furin-like proteases in the Golgi (15). The propeptide of rHuAD10 contains a single cysteine at amino acid 173 which may be regulating the activity of the enzyme through a cysteine switch mechanism as with other metalloproteases (16). Mature HuAD10 is predicted to have 535 amino acids with a calculated M₉ of 59,286. The difference between the calculated M₉ of HuAD10 and its apparent M₉ of 62,000 on SDS-PAGE is likely because of glycosylation, as HuAD10 has four potential sites for N-glycosylation (Fig. 2).

Expression and Purification of rHuAD10—To obtain rHuAD10, a soluble ADAM 10-ig construct was generated and transfected into 293-EBNA cells. Immunoblot analysis with an antibody specific to HuAD10 demonstrated the expression of 98-, 72-, and 62-kDa proteins in the conditioned medium of transfected cells (Fig. 4A). Isolation and characterization of these proteins revealed that TNF processing activity tracked only with the 62-kDa protein. Since the 98-kDa band cross-reacts with an antibody against human Fe, but the 62- and 72-kDa proteins do not (data not shown), we suspect that the proteolytically sensitive Fe hinge region was cleaved from the 62- and 72-kDa proteins by proteases present in 293-EBNA cells or conditioned medium.

A silver-stained gel demonstrating purification of the 62-kDa rHuAD10 to homogeneity is shown in Fig. 4B. rHuAD10 has a specific activity of 1,000 milliunits/mg of protein and an NH₂ terminus that is identical to that of HuAD10 purified from THP-1 cells.

Characterization of rHuAD10—To characterize the proteolytic activity of rHuAD10 versus a partially purified protein preparation (S-Sepharose pool), we assayed for TNF processing activity in the presence of a variety of protease inhibitors. Relative activities, shown in Table II, reveal that the S-Sepharose pool and rHuAD10 have comparable profiles. Results indicate that the serine protease inhibitors had little effect on proteolytic activity, and no significant inhibition was observed with the matrix metalloprotease inhibitors phosphoramidon and TIMP-1. In contrast, known metal chelators (EDTA and 1,10-phenanthroline) inhibited the proteolytic activity in both protein preparations.

The pH optima were examined for both rHuAD10 and partially purified HuAD10 (S-Sepharose pool). Maximum TNF processing activity for both protein preparations was between pH 9 and 10 (data not shown).

Substrate Specificity of rHuAD10—The substrate specificity of rHuAD10 was examined by testing its ability to cleave a variety of peptides with substitutions surrounding the Ala-Val cleavage site. The influence of these substitutions on peptide cleavage is shown in Table III. Alterations at Ala⁷⁶ except for A76S or A76K resulted in a significant decrease in cleavage efficiency. rHuAD10 was better able to tolerate changes at Val¹⁷. Substitutions with Leu, Ile, Arg, Tyr, and Ser had no effect on the ability of rHuAD10 to catalyze peptide cleavage.

We examined peptide sequences spanning the cleavage sites for other proteins known to be shed proteolytically from the cell surface (Table IV). Proteins were chosen based on sequence...
TABLE II
Characterization of rHuAD10

| Inhibitors                | Protease class | rHuAD10 | S-Sepharose pool |
|---------------------------|----------------|---------|------------------|
| Phenylmethylsulfonyl fluoride (10 μM) | Serine, thiol | 0.5 | 5.4 |
| Leupeptin (1 μM)           | Serine         | 3      | 8.6 |
| Aprotinin (0.3 μM)         | Aspartic       | 0      | 0 |
| Pepstatin (1 μM)           | Metallo        | 88     | 36 |
| EDTA (1 mM)                | Metallo        | 100    | 86 |
| 1,10-Phenanthroline (1 mM) | Metallo        | 0      | 0 |
| Phosphoramidon (10 mM)     | Metallo        | 0      | 0 |
| TIMP-1 (20 mM)             | Metallo        | 12     | 12 |

% inhibition

| Inhibitors                | % inhibition | % inhibition |
|---------------------------|--------------|--------------|
| EDTA (2.5 mM)             | 100          | 100          |
| Phosphoramidon (10 mM)    | 100          | 100          |
| TIMP-1 (20 mM)            | 100          | 100          |
| Aprotinin (0.3 μM)        | 100          | 100          |
| Pepstatin (1 μM)          | 0            | 0            |
| Leupeptin (1 μM)          | 0            | 0            |
| Phenylmethylsulfonyl fluoride (10 μM) | 0 | 0 |

TABLE III
Influence of substitutions at the pro-TNF cleavage site

DNP-peptides were synthesized and assayed as described under “Materials and Methods.” rHuAD10 was used as the enzyme source. Samples were incubated for 17 h at 37 °C and assayed in the HPLC assay in place of membrane-bound proteins.

Wild type sequence

| Substitution | % cleavage | Substitution | % cleavage |
|--------------|------------|--------------|------------|
| Wild type    | 100        | V77G         | 0          |
| A76D         | 0          | V77E         | 0          |
| A76L         | 0          | V77P         | 0          |
| A76G         | 11         | V77W         | 49         |
| A76T         | 27         | V77L         | 100        |
| A76S         | 39         | V77I         | 100        |
| A76F         | 63         | V77R         | 100        |
| A76Y         | 95         | V77Y         | 100        |
| A76K         | 100        | V77S         | 100        |
| R78K         | 33         | S79E         | 10         |
| R78T         | 94         | S79T         | 77         |
| R78Q         | 100        | S79R         | 100        |

characterization of rHuAD10

Pro-TNF-α-processing Enzyme

Protease inhibitors were added to each protein preparation at a concentration as recommended by the manufacturer. Activity was assayed by the HPLC peptide assay as described under “Materials and Methods.”

Inhibitors

| Inhibitors                | % inhibition | % inhibition |
|---------------------------|--------------|--------------|
| EDTA (2.5 mM)             | 100          | 100          |
| Phosphoramidon (10 mM)    | 100          | 100          |
| TIMP-1 (20 mM)            | 100          | 100          |
| Aprotinin (0.3 μM)        | 100          | 100          |
| Pepstatin (1 μM)          | 0            | 0            |
| Leupeptin (1 μM)          | 0            | 0            |
| Phenylmethylsulfonyl fluoride (10 μM) | 0 | 0 |

% inhibition

rHuAD10 Will Cleave Pro-TNF to a 17-kDa Species—The purification of HuAD10 from THP-1 cells was based on pooling active fractions as determined by the HPLC peptide assay. To verify that HuAD10 is capable of processing full-length pro-TNF, in vitro translated 35S-labeled pro-TNF was treated with a partially purified HuAD10 (S-Sepharose pool) or rHuAD10. After immunoprecipitation, the reaction mixtures were subjected to SDS-PAGE followed by autoradiography. Fig. 5 shows that both protein preparations cleaved a portion of the 26-kDa pro-TNF to a 17-kDa species corresponding to the molecular weight of soluble mature TNF. It is not known if the processing efficiency of HuAD10 is enhanced when colorolized with pro-TNF on cell membranes.

DISCUSSION

Characterization of rHuAD10 suggests that it has many of the physical and biochemical properties required of a TNF-processing enzyme. Our data indicate that HuAD10 specifically recognizes pro-TNF and is not a general protease involved in the shedding of other cell surface proteins. rHuAD10 processed full-length pro-TNF to a 17-kDa form and cleaved the pro-TNF peptide sequence at a site that corresponds to the authentic NH2 terminus (Val17) of soluble TNF. We also show that substitutions surrounding the cleavage site adversely affect the ability of rHuAD10 to cleave the pro-TNF peptide. Furthermore, rHuAD10 failed to recognize and process peptides spanning the cleavage sites found in other membrane-associated proteins known to be shed proteolytically. These data are consistent with the expectation that a TNF-processing enzyme would have a preferred specificity for its substrate, pro-TNF.

Our inhibitor studies demonstrate that HuAD10 is a functional metalloprotease since it is effectively inhibited by EDTA and 1,10-phenanthroline. Interestingly, two metalloprotease inhibitors, phosphoramidon and TIMP-1, had no effect on TNF processing activity. These results agree with previous experiments that showed an essentially identical inhibition profile for a partially purified TNF-processing enzyme (6).

The protease responsible for TNF processing is likely to be membrane-bound and present on cells that secrete high levels of TNF. Accordingly, THP-1 cells were chosen for purification of the protease. HuAD10 appears to be an integral membrane protein since it fractionated with cell membranes rather than the cytosol. Additionally, it has a classical transmembrane domain. Attempts to verify its presence on the cell surface using fluorescence-activated cell sorting with AMG1961 have not been successful since this antibody does not recognize native HuAD10. We are generating monoclonal antibodies against rHuAD10 to address this question in the future. Northern analysis indicates that HuAD10 mRNA is found in tissues that have detectable TNF mRNA (e.g., spleen, lymph node, peripheral blood leukocytes). Thus, the expression and presumed localization of HuAD10 in cells are consistent with a potential role for this protein in TNF processing. It is not known if HuAD10 has additional functions in tissues that do not express TNF.
Recently, Lunn et al. (9) have shown that a protein preparation enriched in BoAD10 cleaves pro-TNF or a peptide spanning the pro-TNF cleavage site. We have purified rHuAD10 to homogeneity and have shown that this protein possesses high intrinsic activity for cleaving similar substrates. Lunn et al. (9) also reported that 293-EBNA cells processed pro-TNF only when cotransfected with BoAD10 cDNA. We observed that 293-EBNA cells produced substantial amounts of soluble TNF when transfected with pro-TNF. Cotransfection with HuAD10 did not significantly increase soluble TNF above the already high background. We did observe small increases in soluble TNF when insect cells were cotransfected with HuAD10 and pro-TNF, compared with insect cells singly transfected with pro-TNF (data not shown).

Interestingly, other ADAM family members have been recently shown to process pro-TNF (7, 8, 22). Of this group, TACE is the only member that is membrane-bound and found in mammals. Significantly, mutant T-cells lacking TACE were found to be greatly impaired in secreting TNF when stimulated with physiological stimuli. Also yet to be determined is what role, if any, TACE may play in the release of other membrane-bound proteins such as TNF.

TACE and HuAD10 have significant sequence and structural homology and may represent two members of a family of TNF-processing enzymes. We have screened cDNA libraries for novel ADAM family members based on conserved sequences from known members including HuAD10 and have isolated unique ADAM family cDNAs including TACE (data not shown). Recombinant proteins expressed from these cDNAs are now being compared with respect to their relative abilities to process pro-TNF.

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