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(54) Title: REGULATION OF HUMAN CARBOXYPEPTIDASE A

(57) Abstract: Reagents that regulate human carboxypeptidase A and reagents which bind to human carboxypeptidase A gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, asthma and cancer.

REGULATION OF HUMAN CARBOXYPEPTIDASE A

TECHNICAL FIELD OF THE INVENTION

The invention relates to the regulation of human carboxypeptidase A.

BACKGROUND OF THE INVENTION

Carboxypeptidase A1 (EC 3.4.17.1) is a pancreatic exopeptidase. Uratani *et al.*, Biochem J. 2000 Mar 15;346 Pt 3:817-26; Darnis *et al.*, Eur J Biochem. 1999 Feb;259(3):719-25.The proteolytic processing of procarboxypeptidase A1 to carboxypeptidase A1 (CA1) within the pancreatic acinar cell is associated with cellular injury. Grady *et al.*, Am J Physiol. 1998 Nov;275(5 Pt 1):G1010-7. Its expression is up-regulated in the basophilic cells (that express mast cell granule proteases) in the peripheral blood of asthma, allergy, and drug-reactive patients. Li *et al.*, J Immunol. 1998 Nov 1;161(9):5079-86. Because mast cell proteases (Try, Chy, and/or CPA) regulate numerous immunologic and other biologic systems, the expression of these enzymes in a peripheral blood-localized cell in an individual having asthma, allergy, or an allergic drug reaction has important clinical implications.

Carboxypeptidase A1 was also been used as a prodrug-activating enzyme in antibody-directed enzyme prodrug therapy (ADEPT), which has the potential of greatly enhancing antitumor selectivity of cancer therapy by synthesizing chemotherapeutic agents selectively at tumor sites. Wolfe *et al.*, Bioconjug Chem. 1999 Jan-Feb;10(1):38-48. This therapy is based upon targeting a prodrug-activating enzyme to a tumor by attaching the enzyme to a tumor-selective antibody and dosing the enzyme-antibody conjugate systemically. Smith *et al.*, J Biol Chem. 1997 Jun 20;272(25):15804-16; Chong *et al.*, Biochemistry. 2000 Jun 27;39(25):7580-8. After

the enzyme-antibody conjugate is localized to the tumor, the prodrug is then also dosed systemically, and the previously targeted enzyme converts it to the active drug selectively at the tumor.

Because of the clinical importance of carboxypeptidase A1, there is a need in the art to identify additional carboxypeptidase enzymes which can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human carboxypeptidase A. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a carboxypeptidase A polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 61% identical to
the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a carboxypeptidase A polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 61% identical to
the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the carboxypeptidase A polypeptide is detected. A test compound which binds to the carboxypeptidase A polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation.

The agent can work by decreasing the activity of the carboxypeptidase A.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a carboxypeptidase A polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to
the nucleotide sequence shown in SEQ ID NO: 1; and
the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the carboxypeptidase A through interacting with the carboxypeptidase A mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a carboxypeptidase A polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 61% identical to
the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

A carboxypeptidase A activity of the polypeptide is detected. A test compound which increases carboxypeptidase A activity of the polypeptide relative to carboxypeptidase A activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases carboxypeptidase A activity of the polypeptide relative to carboxypeptidase A activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a carboxypeptidase A product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to
the nucleotide sequence shown in SEQ ID NO: 1; and
the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the carboxypeptidase A product is detected. A test compound which binds to the carboxypeptidase A product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a carboxypeptidase A polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to
the nucleotide sequence shown in SEQ ID NO: 1; and
the nucleotide sequence shown in SEQ ID NO: 1.

Carboxypeptidase A activity in the cell is thereby decreased.

The invention thus provides a human carboxypeptidase A that can be used to identify test compounds that may act, for example, as activators or inhibitors at the enzyme's active site. Human carboxypeptidase A and fragments thereof also are useful in raising specific antibodies that can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO: 2).

Fig. 3 shows the amino acid sequence of the protein identified by SwissProt Accession No. P15085|CBP1_HUMAN CARBOXYPEPTIDASE A1 PRECURSOR (SEQ ID NO: 3).

Fig. 4 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 4).

Fig. 5 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 5).

Fig. 6 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 6).

Fig. 7 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 7).

Fig. 8 shows the DNA-sequence encoding a carboxypeptidase A Polypeptide (SEQ ID NO: 8).

Fig. 9 shows the BLASTP-alignment of 356 (SEQ ID NO: 2) against swiss|P15085|CBP1_HUMAN CARBOXYPEPTIDASE A1 PRECURSOR (EC 3.4.17.1).//:trembl|X67318|HSPCBXA1_1 (SEQ ID NO: 3).

Fig. 10 shows the BLASTP - alignment of 356 (SEQ ID NO: 2) against pdb|1PCA|1PCA Procarboxypeptidase a.

Fig. 11 shows the Prosite and BLOCKS search results.

Fig. 12 shows the HMMPFAM - alignment of 356 (SEQ ID NO: 2) against pfam|hmm|Zn_carbOpept Zinc carboxypeptidase.

Fig. 13 shows the HMMPFAM - alignment of 356 (SEQ ID NO: 2) against pfam|hmm|Propep_M14 Carboxypeptidase activation peptide.

Fig. 14 shows the BLASTP-alignment of 356 against trembl|M75106|HSPCPBX_1.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide being selected from the group consisting of:

a) a polynucleotide encoding a carboxypeptidase A polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 61% identical to the amino acid sequence shown in SEQ ID NO: 2; and the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a carboxypeptidase A polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a carboxypeptidase A polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a carboxypeptidase A polypeptide.

Furthermore, it has been discovered by the present applicant that a novel carboxypeptidase A, particularly a human carboxypeptidase A, can be used in therapeutic methods to treat asthma or cancer. Human carboxypeptidase A comprises the amino acid sequence shown in SEQ ID NO: 2. A coding sequence for human carboxypeptidase A is shown in SEQ ID NO: 1. Related ESTs (SEQ ID NOS: 4-8) are expressed in testis, pooled library (fetal lung NbHL19W, testis NHT, and B-cell NCI_CGAP_GCB1).

Human carboxypeptidase A is 60% identical over 418 amino acids to swiss|P15085|CBP1_HUMAN CARBOXYPEPTIDASE A1 PRECURSOR (EC 3.4.17.1).//:trembl|X67318|HSPCBXA1_1 (SEQ ID NO: 3) (Fig. 9). Human

carboxypeptidase A of the invention is expected to be useful for the same purposes as previously identified carboxypeptidase A enzymes. Human carboxypeptidase A is believed to be useful in therapeutic methods to treat disorders such as asthma and cancer. Human carboxypeptidase A also can be used to screen for human carboxypeptidase A activators and inhibitors.

Polypeptides

Human carboxypeptidase A polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 436 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof, as defined below. A carboxypeptidase A polypeptide of the invention therefore can be a portion of a carboxypeptidase A protein, a full-length carboxypeptidase A protein, or a fusion protein comprising all or a portion of a carboxypeptidase A protein.

Biologically Active Variants

Human carboxypeptidase A polypeptide variants that are biologically active, *e.g.*, retain a carboxypeptidase activity, also are carboxypeptidase A polypeptides. Preferably, naturally or non-naturally occurring carboxypeptidase A polypeptide variants have amino acid sequences which are at least about 61, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, 98, or 99% identical to the amino acid sequence shown in SEQ ID NO: 2 or a fragment thereof. Percent identity between a putative carboxypeptidase A polypeptide variant and an amino acid sequence of SEQ ID NO: 2 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino

acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a carboxypeptidase A polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active carboxypeptidase A polypeptide can readily be determined by assaying for carboxypeptidase activity, as described for example, in Darnis *et al.*, Eur J Biochem 1999 Feb;259(3):719-25.

Fusion Proteins

Fusion proteins are useful for generating antibodies against carboxypeptidase A polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins that interact with portions of a carboxypeptidase A polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A carboxypeptidase A polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 436 contiguous amino acids of SEQ ID NO: 2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length carboxypeptidase A protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the carboxypeptidase A polypeptide-encoding sequence and the heterologous protein sequence, so that the carboxypeptidase A polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NO: 1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human carboxypeptidase A polypeptide can be obtained using carboxypeptidase A polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of carboxypeptidase A polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A carboxypeptidase A polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a carboxypeptidase A polypeptide. A coding sequence for human carboxypeptidase A is shown in SEQ ID NO: 1. This sequence is on chromosome 7 and next to the the known Carboxypeptidase A1 (Swiss-prot entry:CBP1_HUMAN).

Degenerate nucleotide sequences encoding human carboxypeptidase A polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, 98, or 99% identical to the nucleotide sequence shown in SEQ ID NO: 1 or its complement also are carboxypeptidase A polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of carboxypeptidase A polynucleotides that encode biologically active carboxypeptidase A polypeptides also are carboxypeptidase A polynucleotides. Polynucleotide fragments comprising at least 8, 9, 10, 11, 12, 15, 20, or 25 contiguous nucleotides of SEQ ID NO: 1 or its complement also are carboxypeptidase A polynucleotides. These fragments can be used, for example, as hybridization probes or as antisense oligonucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the carboxypeptidase A polynucleotides described above also are carboxypeptidase A polynucleotides. Typically, homologous carboxypeptidase A polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known carboxypeptidase A polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the carboxypeptidase A polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of carboxypeptidase A polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al., J. Mol. Biol. 81*, 123 (1973). Variants of human carboxypeptidase A polynucleotides or carboxypeptidase A polynucleotides of other species can therefore be identified by hybridizing a putative homologous carboxypeptidase A polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO: 1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to carboxypeptidase A polynucleotides or their complements following stringent hybridization and/or wash conditions also are carboxypeptidase A polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a carboxypeptidase A polynucleotide having a nucleotide sequence shown in SEQ ID NO: 1 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l),$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A carboxypeptidase A polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a poly-

nucleotide can be used to obtain isolated carboxypeptidase A polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises carboxypeptidase A nucleotide sequences. Isolated polynucleotides are in preparations that are free or at least 70, 80, or 90% free of other molecules.

Human carboxypeptidase A cDNA molecules can be made with standard molecular biology techniques, using carboxypeptidase A mRNA as a template. Human carboxypeptidase A cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize carboxypeptidase A polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a carboxypeptidase A polypeptide having, for example, an amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are tran-

scribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an

oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) that are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA that might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Human carboxypeptidase A polypeptides can be obtained, for example, by purification from human cells, by expression of carboxypeptidase A polynucleotides, or by direct chemical synthesis.

Protein Purification

Human carboxypeptidase A polypeptides can be purified from any cell that expresses the enzyme, including host cells that have been transfected with carboxypeptidase A expression constructs. A purified carboxypeptidase A polypeptide is separated from other compounds that normally associate with the carboxypeptidase A polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity

chromatography, and preparative gel electrophoresis. A preparation of purified carboxypeptidase A polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

To express a carboxypeptidase A polynucleotide, the polynucleotide can be inserted into an expression vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods that are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding carboxypeptidase A polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a carboxypeptidase A polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can

vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a carboxypeptidase A polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the carboxypeptidase A polypeptide. For example, when a large quantity of a carboxypeptidase A polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the carboxypeptidase A polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione.

Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding carboxypeptidase A polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a carboxypeptidase A polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding carboxypeptidase A polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of carboxy A

polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which carboxypeptidase A polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express carboxypeptidase A polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding carboxypeptidase A polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus that is capable of expressing a carboxypeptidase A polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding carboxypeptidase A polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a carboxypeptidase A polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals

(including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed carboxypeptidase A polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express carboxypeptidase A polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced carboxypeptidase A sequences. Resistant clones of stably

transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*⁻ or *aprt*⁻ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the carboxypeptidase A polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a carboxypeptidase A polypeptide is inserted within a marker gene sequence, transformed cells containing sequences that encode a carboxypeptidase A polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a

sequence encoding a carboxypeptidase A polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the carboxypeptidase A polynucleotide.

Alternatively, host cells which contain a carboxypeptidase A polynucleotide and which express a carboxypeptidase A polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques that include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a carboxypeptidase A polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a carboxypeptidase A polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a carboxypeptidase A polypeptide to detect transformants that contain a carboxypeptidase A polynucleotide.

A variety of protocols for detecting and measuring the expression of a carboxypeptidase A polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a carboxypeptidase A polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding carboxypeptidase A polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a carboxypeptidase A polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a carboxypeptidase A polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode carboxypeptidase A polypeptides can be designed to contain signal sequences which direct secretion of soluble carboxypeptidase A polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound carboxypeptidase A polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a carboxypeptidase A polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification

facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the carboxypeptidase A polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a carboxypeptidase A polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the carboxypeptidase A polypeptide from the fusion protein. Vectors that contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a carboxypeptidase A polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a carboxypeptidase A polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of carboxypeptidase A polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic carboxypeptidase A polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the carboxypeptidase A polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce carboxypeptidase A polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life that is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter carboxypeptidase A polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a carboxypeptidase A polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a carboxypeptidase A polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a carboxypeptidase A polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody that specifically binds to the immunogen.

Typically, an antibody which specifically binds to a carboxypeptidase A polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to carboxypeptidase A polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a carboxypeptidase A polypeptide from solution.

Human carboxypeptidase A polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a carboxypeptidase A polypeptide can be conjugated to a

carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies that specifically bind to a carboxypeptidase A polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies that specifically bind to a carboxypeptidase A polypeptide

can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies that specifically bind to carboxypeptidase A polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to carboxypeptidase A polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as

disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a carboxypeptidase A polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of carboxypeptidase A gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such

alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of carboxypeptidase A gene expression can be obtained by designing antisense oligonucleotides that will form duplexes to the control, 5', or regulatory regions of the carboxypeptidase A gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a carboxypeptidase A polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a carboxypeptidase A polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent carboxypeptidase A nucleotides, can provide sufficient targeting specificity for carboxypeptidase A mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of

mismatching which will be tolerated between a particular antisense oligonucleotide and a particular carboxypeptidase A polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a carboxypeptidase A polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, inter-nucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a carboxypeptidase A polynucleotide can be used to generate ribozymes that will specifically bind to mRNA transcribed from the carboxypeptidase A polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a carboxypeptidase A RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate carboxypeptidase A RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease carboxypeptidase A expression. Alternatively, if it is desired that the cells stably retain the DNA

construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors that induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human carboxypeptidase A. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, asthma and cancer. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human carboxypeptidase A gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human carboxypeptidase A. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human carboxypeptidase A. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human carboxypeptidase A gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds that bind to or modulate the activity of a carboxypeptidase A polypeptide or a carboxypeptidase A polynucleotide. A test compound preferably binds to a carboxypeptidase A polypeptide or polynucleotide. More preferably, a test compound decreases or increases carboxypeptidase activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J.*

Med. Chem. 37, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to carboxypeptidase A polypeptides or polynucleotides or to affect carboxypeptidase A activity or carboxypeptidase A gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule that binds to and occupies, for example, the active site of the carboxypeptidase A polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the carboxypeptidase A polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemilumi-

nescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound that is bound to the carboxypeptidase A polypeptide can then be accomplished, for example, by direct counting of radio-emmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a carboxypeptidase A polypeptide can be determined without labeling either of the interactants. For example, a micro-physiometer can be used to detect binding of a test compound with a carboxy-peptidase A polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a carboxypeptidase A polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a carboxypeptidase A poly-peptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a carboxypeptidase A polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins

which bind to or interact with the carboxypeptidase A polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a carboxypeptidase A polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein that interacts with the carboxypeptidase A polypeptide.

It may be desirable to immobilize either the carboxypeptidase A polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the carboxypeptidase A polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test

compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a carboxypeptidase A polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the carboxypeptidase A polypeptide is a fusion protein comprising a domain that allows the carboxypeptidase A polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed carboxypeptidase A polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a carboxypeptidase A polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated carboxypeptidase A polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a carboxypeptidase A polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active

site of the carboxypeptidase A polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the carboxypeptidase A polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the carboxypeptidase A polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a carboxypeptidase A polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a carboxypeptidase A polypeptide or polynucleotide can be used in a cell-based assay system. A carboxypeptidase A polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a carboxypeptidase A polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the carboxypeptidase activity of a human carboxypeptidase A polypeptide. Carboxypeptidase activity can be measured, for example, as described in Darnis *et al.*, Eur J Biochem 1999 Feb;259(3):719-25.

Enzyme assays can be carried out after contacting either a purified carboxypeptidase A polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound that decreases a carboxypeptidase activity of a carboxypeptidase A polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing carboxypeptidase

A activity. A test compound which increases a carboxypeptidase activity of a human carboxypeptidase A polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human carboxypeptidase A activity.

Gene Expression

In another embodiment, test compounds that increase or decrease carboxypeptidase A gene expression are identified. A carboxypeptidase A polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the carboxypeptidase A polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of carboxypeptidase A mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a carboxypeptidase A polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a carboxypeptidase A polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell that expresses a carboxypeptidase A polynucleotide can be used in a cell-based assay system. The carboxypeptidase A polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions that can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a carboxypeptidase A polypeptide, carboxypeptidase A polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a carboxypeptidase A polypeptide, or mimetics, activators, or inhibitors of a carboxypeptidase A polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using

pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be

placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

Human carboxypeptidase A can be regulated to treat asthma and cancer. Allergy is a complex process in which environmental antigens induce clinically adverse reactions. The inducing antigens, called allergens, typically elicit a specific IgE response and, although in most cases the allergens themselves have little or no intrinsic toxicity, they induce pathology when the IgE response in turn elicits an IgE-dependent or T cell-dependent hypersensitivity reaction. Hypersensitivity reactions can be local or systemic and typically occur within minutes of allergen exposure in individuals who have previously been sensitized to an allergen. The hypersensitivity reaction of allergy develops when the allergen is recognized by IgE antibodies bound to specific receptors on the surface of effector cells, such as mast cells, basophils, or eosinophils, which causes the activation of the effector cells and the release of mediators that produce the acute signs and symptoms of the reactions. Allergic diseases include asthma, allergic rhinitis (hay fever), atopic dermatitis, and anaphylaxis.

Asthma is though to arise as a result of interactions between multiple genetic and environmental factors and is characterized by three major features: 1) intermittent and reversible airway obstruction caused by bronchoconstriction, increased mucus production, and thickening of the walls of the airways that leads to a narrowing of the airways, 2) airway hyperresponsiveness caused by a decreased control of airway caliber, and 3) airway inflammation. Certain cells are critical to the inflammatory reaction of asthma and they include T cells and antigen presenting cells, B cells that produce IgE, and mast cells, basophils, eosinophils, and other cells that bind IgE. These effector cells accumulate at the site of allergic reaction in the airways and release toxic products that contribute to the acute pathology and eventually to the

tissue destruction related to the disorder. Other resident cells, such as smooth muscle cells, lung epithelial cells, mucus-producing cells, and nerve cells may also be abnormal in individuals with asthma and may contribute to the pathology. While the airway obstruction of asthma, presenting clinically as an intermittent wheeze and shortness of breath, is generally the most pressing symptom of the disease requiring immediate treatment, the inflammation and tissue destruction associated with the disease can lead to irreversible changes that eventually make asthma a chronic disabling disorder requiring long-term management.

Despite recent important advances in our understanding of the pathophysiology of asthma, the disease appears to be increasing in prevalence and severity (Gergen and Weiss, *Am. Rev. Respir. Dis. 146*, 823-24, 1992). It is estimated that 30-40% of the population suffer with atopic allergy, and 15% of children and 5% of adults in the population suffer from asthma (Gergen and Weiss, 1992). Thus, an enormous burden is placed on our health care resources. However, both diagnosis and treatment of asthma are difficult. The severity of lung tissue inflammation is not easy to measure and the symptoms of the disease are often indistinguishable from those of respiratory infections, chronic respiratory inflammatory disorders, allergic rhinitis, or other respiratory disorders. Often, the inciting allergen cannot be determined, making removal of the causative environmental agent difficult. Current pharmacological treatments suffer their own set of disadvantages. Commonly used therapeutic agents, such as beta agonists, can act as symptom relievers to transiently improve pulmonary function, but do not affect the underlying inflammation. Agents that can reduce the underlying inflammation, such as anti-inflammatory steroids, can have major drawbacks that range from immunosuppression to bone loss (Goodman and Gilman's THE PHARMACOLOGIC BASIS OF THERAPEUTICS, Seventh Edition, MacMillan Publishing Company, NY, USA, 1985). In addition, many of the present therapies, such as inhaled corticosteroids, are short-lasting, inconvenient to use, and must be used often on a regular basis, in some cases for life, making failure of patients to

comply with the treatment a major problem and thereby reducing their effectiveness as a treatment.

Because of the problems associated with conventional therapies, alternative treatment strategies have been evaluated. Glycophorin A (Chu and Sharom, *Cell. Immunol. 145*, 223-39, 1992), cyclosporin (Alexander *et al.*, *Lancet 339*, 324-28, 1992), and a nonapeptide fragment of IL-2 (Zav'yalov *et al.*, *Immunol. Lett. 31*, 285-88, 1992) all inhibit interleukin-2 dependent T lymphocyte proliferation; however, they are known to have many other effects. For example, cyclosporin is used as a immunosuppressant after organ transplantation. While these agents may represent alternatives to steroids in the treatment of asthmatics, they inhibit interleukin-2 dependent T lymphocyte proliferation and potentially critical immune functions associated with homeostasis. Other treatments that block the release or activity of mediators of bronchochonstriction, such as cromones or anti-leukotrienes, have recently been introduced for the treatment of mild asthma, but they are expensive and not effective in all patients and it is unclear whether they have any effect on the chronic changes associated with asthmatic inflammation. What is needed in the art is the identification of a treatment that can act in pathways critical to the development of asthma that both blocks the episodic attacks of the disorder and preferentially dampens the hyperactive allergic immune response without immunocompromising the patient.

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-

resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a carboxypeptidase A polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects carboxypeptidase A activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce carboxypeptidase A activity. The reagent preferably binds to an expression product of a human carboxypeptidase A gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells that have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods that are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE

TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases carboxypeptidase A activity relative to the carboxypeptidase A activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For

administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides that express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a carboxypeptidase A gene or the activity of a carboxypeptidase A polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a carboxypeptidase A gene or the activity of a carboxypeptidase A polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to carboxypeptidase A-specific mRNA, quantitative RT-PCR, immunologic detection of a carboxypeptidase A polypeptide, or measurement of carboxypeptidase A activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human carboxypeptidase A also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences that encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding carboxypeptidase A in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions

according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a carboxypeptidase A also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of carboxypeptidase-like enzyme activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-carboxypeptidase-like enzyme polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and the carboxypeptidase-like enzyme activity is measured

according to the o-phthaldialdehyde [OPA] method which is based on detection of a free amino group. At 25°C, 50 µl of the cell extract is added to 1 ml of a 0,5 mM carbobenzoxy (Z)-Ala-Glu solution in 20 mM sodium acetate buffer (pH 4,5). The release of free glutamic acid is monitored by using OPA and dithiothreitol (18). One unit of activity is defined as the quantity of enzyme which liberated 1 µmol of glutamic acid per min under the conditions described above for the second method. It is shown that the polypeptide of SEQ ID NO: 2 has a carboxypeptidase-like enzyme activity.

EXAMPLE 2

Expression of recombinant human carboxypeptidase A

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human carboxypeptidase A polypeptides in yeast. The carboxypeptidase A-encoding DNA sequence is derived from SEQ ID NO: 1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter

tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human carboxypeptidase A polypeptide is obtained.

EXAMPLE 3

Identification of test compounds that bind to carboxypeptidase A polypeptides

Purified carboxypeptidase A polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human carboxypeptidase A polypeptides comprise the amino acid sequence shown in SEQ ID NO: 2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a carboxypeptidase A polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound that increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a carboxypeptidase A polypeptide.

EXAMPLE 4

Identification of a test compound which decreases carboxypeptidase A gene expression

A test compound is administered to a culture of human cells transfected with a carboxypeptidase A expression construct and incubated at 37°C for 10 to 45 minutes.

A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 μg total RNA and hybridized with a ^{32}P-labeled carboxypeptidase A-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO: 1. A test compound that decreases the carboxypeptidase A-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of carboxypeptidase A gene expression.

EXAMPLE 5

Identification of a test compound which decreases carboxypeptidase A activity

A test compound is administered to a culture of human cells transfected with a carboxypeptidase A expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control. Carboxypeptidase activity is measured using the method of Darnis *et al.*, Eur J Biochem 1999 Feb;259(3):719-25.

A test compound which decreases the carboxypeptidase activity of the carboxypeptidase A relative to the carboxypeptidase activity in the absence of the test compound is identified as an inhibitor of carboxypeptidase A activity.

EXAMPLE 6

Tissue-specific expression of carboxypeptidase A

The qualitative expression pattern of carboxypeptidase A in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). To demonstrate that carboxypeptidase A is involved in cancer, expression is determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate), NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

To demonstrate that carboxypeptidase A is involved in the disease process of asthma, the following whole body panel is screened to show predominant or relatively high expression in lung or immune tissues: brain, heart, kidney, liver, lung, trachea, bone marrow, colon, small intestine, spleen, stomach, thymus, mammary gland, skeletal muscle, prostate, testis, uterus, cerebellum, fetal brain, fetal liver, spinal cord, placenta, adrenal gland, pancreas, salivary gland, thyroid, peripheral blood leukocytes, lymph node, and tonsil. Once this is established, the following lung and immune system cells are screened to localize expression to particular cell subsets: lung microvascular endothelial cells, bronchial/trachial epithelial cells, bronchial/-trachial smooth muscle cells, lung fibroblasts, T cells (T helper 1 subset, T helper 2 subset, NKT cell subset, and cytotoxic T lymphocytes), B cells, mononuclear cells (monocytes and macrophages), mast cells, eosinophils, neutrophils, and dendritic cells. As a final step, the expression of carboxypeptidase A in cells derived from

normal individuals with the expression of cells derived from asthmatic individuals is compared.

Quantitative expression profiling. Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res. 6*, 986-94, 1996, and Gibson *et al.*, *Genome Res. 6*, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted

from autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty μg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/μl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/μl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM $MgCl_2$; 50 mM NaCl; and 1 mM DTT.

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:-isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

Fifty μg of each RNA from the autoptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200ng/μL. Reverse transcription is carried out with 2.5μM of random hexamer primers.

TaqMan quantitative analysis. Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; the probe can be labeled at the 5' end FAM (6-carboxy-fluorescein) and at the 3' end with TAMRA (6-carboxy-tetramethyl-rhodamine). Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix

(from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 µl.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

EXAMPLE 7

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000 cells per milliliter in a volume of 0.5 ml and kept at 37°C in a 95% air/5%CO_2 atmosphere.

Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO: 1 is used as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate

buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleotides are added to the culture medium at a concentration of 10 μM once per day for seven days.

The addition of the test oligonucleotide for seven days results in significantly reduced expression of human serine palmitoyltransferase as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of human serine palmitoyltransferase has an anti-proliferative effect on cancer cells.

EXAMPLE 8

In vivo testing of compounds/target validation

1. Acute Mechanistic Assays

1.1. Reduction in Mitogenic Plasma Hormone Levels

This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of interest. If the normal circulating levels of the hormone are too low

and/or variable to provide consistent results, the level of the hormone may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

1.2. Hollow Fiber Mechanism of Action Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these may include assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2. Subacute Functional *In Vivo* Assays

2.1. Reduction in Mass of Hormone Dependent Tissues

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a

predetermined schedule and for a predetermined duration (i.e., 1 week). At termination of the study, animals are weighed, the target organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

2.2. Hollow Fiber Proliferation Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell proliferation is determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2.3. Anti-angiogenesis Models

2.3.1. Corneal Angiogenesis

Hydron pellets with or without growth factors or cells are implanted into a micropocket surgically created in the rodent cornea. Compound administration may be systemic or local

(compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. Image analysis data is evaluated by measuring the area of neovascularization (in pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. Matrigel Angiogenesis

Matrigel, containing cells or growth factors, is injected sub-cutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at predetermined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, special staining for endothelial surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

3. Primary Antitumor Efficacy

3.1. Early Therapy Models

3.1.1. Subcutaneous Tumor

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor burden. Body weights and tumor measurements are recorded 2-3 times weekly. Mean net body and tumor weights are calculated for each data collection day. Anti-tumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

3.1.2. Intraperitoneal/Intracranial Tumor Models

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is

indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment.

3.2. Established Disease Model

Tumor cells or fragments are implanted subcutaneously and grown to the desired size for treatment to begin. Once at the predetermined size range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value≤ 0.05 compared to the vehicle control group.

3.3. Orthotopic Disease Models

3.3.1. Mammary Fat Pad Assay

Tumor cells or fragments, of mammary adenocarcinoma

origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value≤ 0.05 compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. Intraprostatic Assay

Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions throught e abdomen and skin are closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to

the control group in the experiment.

3.3.3. Intrabronchial Assay

Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.4. Intracecal Assay

Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

4.1. Spontaneous Metastasis

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or

liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

4.2. Forced Metastasis

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number

of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

5

CLAIMS

1. An isolated polynucleotide being selected from the group consisting of:

a) a polynucleotide encoding a carboxypeptidase A polypeptide comprising an amino acid sequence selected form the group consisting of:

amino acid sequences which are at least about 61% identical to the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a carboxypeptidase A polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a carboxypeptidase A polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d) and encodes a carboxypeptidase A polypeptide.

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified carboxypeptidase A polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a carboxypeptidase A polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the carboxypeptidase A polypeptide; and

b) recovering the carboxypeptidase A polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a carboxypeptidase A polypeptide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a carboxypeptidase A polypeptide of claim 4 comprising the steps of:

contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the carboxypeptidase A polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a carboxypeptidase A, comprising the steps of:

contacting a test compound with any carboxypeptidase A polypeptide encoded by any polynucleotide of claim1;

detecting binding of the test compound to the carboxypeptidase A polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a carboxypeptidase A.

11. A method of screening for agents which regulate the activity of a carboxypeptidase A, comprising the steps of:

contacting a test compound with a carboxypeptidase A polypeptide encoded by any polynucleotide of claim 1; and

detecting a carboxypeptidase A activity of the polypeptide, wherein a test compound which increases the carboxypeptidase A activity is identified as a potential therapeutic agent for increasing the activity of the carboxypeptidase A, and wherein a test compound which decreases the carboxypeptidase A activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the carboxypeptidase A.

12. A method of screening for agents which decrease the activity of a carboxypeptidase A, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound

which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of carboxypeptidase A.

13. A method of reducing the activity of carboxypeptidase A, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any carboxypeptidase A polypeptide of claim 4, whereby the activity of carboxypeptidase A is reduced.

14. A reagent that modulates the activity of a carboxypeptidase A polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a carboxypeptidase A in a disease.

17. Use of claim 16 wherein the disease is asthma or cancer.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

19. The cDNA of claim 18 which comprises SEQ ID NO: 1.

20. The cDNA of claim 18 which consists of SEQ ID NO: 1.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO: 1.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO: 1.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO: 2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO: 2.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and

isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO: 1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1; and

instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human carboxypeptidase A, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 61% identical to the amino acid sequence shown in SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human carboxypeptidase A.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human carboxypeptidase A, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 61% identical to the amino acid sequence shown in SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human carboxypeptidase A, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human carboxypeptidase A.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human carboxypeptidase A, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO: 1; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human carboxypeptidase A.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human carboxypeptidase A, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1, whereby the activity of a human carboxypeptidase A is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and

a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1; and

a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and

a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO: 1.

68. A method of treating a carboxypeptidase A dysfunction related disease, wherein the disease is selected from asthma or cancer comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human carboxypeptidase A, whereby symptoms of the carboxypeptidase A disfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

atgcagggca cccctggagg cgggacgcgc cctgggccat cccccgtgga caggcggaca
ctcctggtct tcagctttat cctggcagca gctttgggcc aaatgaattt cacaggggac
caggttcttc gagtcctggc caaagatgag aagcagcttt cacttctcgg ggatctggag
ggcctgaaac cccagaaggt ggacttctgg cgtggcccag ccaggcccag cctccctgtg
gatatgagag ttcctttctc tgaactgaaa gacatcaaag cttatctgga gtctcatgga
cttgcttaca gcatcatgat aaaggacatc caggtgctgc tggatgagga aagacaggcc
atggcgaaat cccgccggct ggagcgcagc accaacagct tcagttactc atcataccac
accctggagg agatatatag ctggattgac aactttgtaa tggagcattc cgatattgtc
tcaaaaattc agattggcaa cagctttgaa aaccagtcca ttcttgtcct gaagttcagc
actggaggtt ctcggcaccc agccatctgg attgacactg gaattcactc ccgggagtgg
atcacccatg ccaccggcat ctggactgcc aataagattg tcagtgatta tggcaaagac
cgtgtcctga cagacatact gaatgccatg gacatcttca tagagctcgt cacaaaccct
gatgggtttg cttttaccca cagcatgaac cgcttatggc ggaagaacaa gtccatcaga
cctggaatct tctgcatcgg cgtggatctc aacaggaact ggaagtcggg ttttggagga
aatggttcta acagcaaccc ctgctcagaa acttatcacg ggccctcccc tcagtcggag
ccggaggtgg ctgccatagt gaacttcatc acagcccatg gcaacttcaa ggctctgatc
tccatccaca gctactctca gatgcttatg tacccttacg gccgattgct ggagcccgtt
tcaaatcaga gggagttgta cgatcttgcc aaggatgcgg tggaggcctt gtataaggtc
catgggatcg agtacatttt tggcagcatc agcaccaccc tctatgtggc cagtgggatc
accgtcgact gggcctatga cagtggcatc aagtacgcct tcagctttga gctccgggac
actgggcagt atggcttcct gctgccggcc acacagatca tccccacggc ccaggagacg
tggatggcgc ttcggaccat catggagcac accctgaatc acccctacta g

Fig. 2

MQGTPGGGTR PGPSPVDRRT LLVFSFILAA ALGQMNFTGD QVLRVLAKDE KQLSLLGDLE
GLKPQKVDFW RGPARPSLPV DMRVPFSELK DIKAYLESHG LAYSIMIKDI QVLLDEERQA
MAKSRRLERS TNSFSYSSYH TLEEIYSWID NFVMEHSDIV SKIQIGNSFE NQSILVLKFS
TGGSRHPAIW IDTGIHSREW ITHATGIWTA NKIVSDYGKD RVLTDILNAM DIFIELVTNP
DGFAFTHSMN RLWRKNKSIR PGIFCIGVDL NRNWKSGFGG NGSNSNPCSE TYHGPSPQSE
PEVAAIVNFI TAHGNFKALI SIHSYSQMLM YPYGRLLEPV SNQRELYDLA KDAVEALYKV
HGIEYIFGSI STTLYVASGI TVDWAYDSGI KYAFSFELRD TGQYGFLLPA TQIIPTAQET
WMALRTIMEH TLNHPY

Fig. 3

MRGLLVLSVL LGAVFGKEDF VGHQVLRISV ADEAQVQKVK ELEDLEHLQL DFWRGPAHPG
SPIDVRVPFP SIQAVKIFLE SHGISYETMI EDVQSLLDEE QEQMFAFRSR ARSTDTFNYA
TYHTLEEIYD FLDLLVAENP HLVSKIQIGN TYEGRPIYVL KFSTGGSKRP AIWIDTGIHS
REWVTQASGV WFAKKITQDY GQDAAFTAIL DTLDIFLEIV TNPDGFAFTH STNRMWRKTR
SHTAGSLCIG VDPNRNWDAG FGLSGASSNP CSETYHGKFA NSEVEVKSIV DFVKDHGNIK
AFISIHSYSQ LLMYPYGYKT EPVPDQDELD QLSKAAVTAL ASLYGTKFNY GSIIKAIYQA
SGSTIDWTYS QGIKYSFTFE LRDTGRYGFL LPASQIIPTA KETWLALLTI MEHTLNHPY

Fig. 4

CCTTGGGGAGAAGGATGGAGCCTCCTGCCCTCAGTCGTGTTGCTAGTAGGGGTGATTCAG
GGTGTGCTCCATGATGGTCCGAAGCGCCATCCACGTCTCCTGGGCCGTGGGGATGATCTG
TGTGGCCGGCAGCAGGAAGCCATACTGCCCAGTGTCCCGGAGCTCAAAGCTGAAGGCGTA
CTTGATGCCACTGTCATAGGCCCAGTCGACGGTGATCCCACTGGCCACATAGAGGGTGGT
GCTGATGCTGCCAAAAATGTACTCGATCCCATGGACCTTATACAAGGCCTCCACCGCATC
CTTGGCAAGATCGTACTGCAAGATGATCATTGGCGGGGTGAAAGGCAGG

Fig. 5

TTTTTTGCCTGTTCAAACTTGTATTTATTTTGTAAGAGGTCGGGATGAGGGCATGGGGAT
GGGGATGCATAACTTGGGTTTCGGGAGGAGCCACAGACCTTGGGGAGAAGGATGGAGCCT
CCTGCCCTCAGTCGTGCTGCTAGTAGGGGTGATTCAGGGTGTGCTCCATGATGGTCCGAA
GCGCCATCCACGTCTCCTGGGCCGTGGGGATGATCTGTGTGGCCGGCAGCAGGAAGCCAT
ACTGCCCAGTGTCCCGGAGCTCAAAGCTGAAGGCGTACTTGATGCCACTGTCATAGGCCC
AGTCGACGGTGATCCCACTGGCCACATAGAGGGTGGTGCTGATGCTGCCAAAAATGTACT
CGATCCCATGGACCTTATACAAGG

Fig. 6

TTTCCTGTCTAAACTTGTATTTATTTTCTAAGAGGTCGGGATGAGGGCATGGGGATGGGG
ATGCATAACTTGGGTTTCGGGAGGAGCCACAGACCTTGGGGAGAAGGATGGAGCCTCCTG
CCCTCAGTCGTGCTGCTAGTAGGGGTGATTCAGGGTGTGCTCCATGATGGTCCGAAGCGC
CATCCACGTCTCCTGGGCCGTGGGGATGATCTGTGTGGCCGGCAGCAGGAAGCCATACTG
CCCAGTGTCCCGGAGCTCAAAGCTGAAGGCGTACTTGATGCCACTGTCATAGGCCCAGTC
GACGGTGATCCCACTGGCCACATAGAGGGTGGTGCTGATGCTGCCAAAAATGTACTCGAT
CCCATGGACCTT

Fig. 7

TTTTCCTGTTCAAACTTGTATTTATTTTCTAAGAGGTCGGGATGAGGGCATGGGGATGGG
GATGCATAACTTGGGTTTCGGGAGGAGCCACAGACCTTGGGGAGAAGGATGGAGCCTCCT
GCCCTCAGTCGTGCTGCTAGTAGGGGTGATTCAGGGTGTGCTCCATGATGGTCCGAAGCG
CCATCCACGTCTCCTGGGCCGTGGGGATGATCTGTGTGGCCGGCAGCAGGAAGCCATACT
GCCCAGTGTCCCGGAGCTCAAAGCTGAAGGCGTACTTGATGCCACTGTCATAGGCCCAGT
CGACGGTGATCCCACTGGCCACATCAACTCCCTCTGATTTGAAACGGGCTCCAGCAATCG
GCCGTAAGGGTACATAAGCATCTGAGAGTAGCTGTGGATGGAGATCAGAGCCTTGAAGTT
GCCATGGGCTGTGATGAAGTTCACTATGGCAGCCACC

Fig. 8

TTTTTTTTTTTTTTTTTTGCCTGTTCAAACTTGTATTTATTTTCTAAAAGGTCGGGATGA
GGGCATGGGGATGGGGATGCATAACTTGGGTTTCGGGAGGAGCCACAAACCTTGGGGAAA
AGGATGGAGCCTCCTGCCCTCAATCGTGCTGCTAGTAGGGGTGATTCAGGGTGTGCTCCA
TGATGGTCCGAAACGCCATCCACGTCTCCTGGGCCGTGGGGATGATCTGTGTGGCCGGCA
GCAGGAAGCCATACTGCCCAGTGTCCCGGAGCTCAAAGCTGAAGGCGTACTTGATGCCAC
TGTCATAGGCCCAGTCNACGGTGATCCCACTGGCCACATANAGGGTGGTGCTGATGCTGC
CAAAAATGTACTCGATCCCATGGACCTTATACAAGGCCTCCACCGCATCCTT

Fig. 9

BLASTP - alignment of 356 against swiss|P15085|CBP1_HUMAN

CARBOXYPEPTIDASE A1 PRECURSOR (EC 3.4.17.1).//:trembl|X67318|HSPCBXA1_1 product: "carboxypeptidase a"; H.sapiens mRNA for procarboxypeptidase A1 //:trembl|A51043|A51043_1 unnamed ORF; Sequence 1 from Patent WO9616179. //:gp|A51043|2303825 unnamed ORF; Sequence 1 from Patent WO9616179. //:gp|X67318|35330 product: "carboxypeptidase a"; H.sapiens mRNA for procarboxypeptidase A1.

This hit is scoring at : 5e-152 (expectation value)

Alignment length (overlap) : 418

Identities : 60 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Database searched : nrdb

```
Q:     19 RTLLVFSFILAAALGQMNFTGDQVLRVLAKDEKQLSLLGDLEGLKPQKVDFWRGPARPSL
          R LLV.S.:L.A..G: :F.G.QVLR: ..DE.Q:. : :LE.L:  ::DFWRGPA.P.
H:      2 RGLLVLSVLLGAVFGKEDFVGHQVLRISVADEAQVQKVKELEDLEHLQLDFWRGPAHPGS
          PVDMRVPFSELKDIKAYLESHGLAYSIMIKDIQVLLDEERQAMAKSRRLERSTNSFSYSS
          P:D:RVPF..:: :K.:LESHG::Y..MI:D:Q LLDEE::.M . R. .RST::F:Y::
          PIDVRVPFPSIQAVKIFLESHGISYETMIEDVQSLLDEEQEQMFAFRSRARSTDTFNYAT
          YHTLEEIYSWIDNFVMEHSDIVSKIQIGNSFENQSILVLKFSTGGSRHPAIWIDTGIHSR
          YHTLEEIY.::D .V.E:..:VSKIQIGN::E.:.I.VLKFSTGGS:.PAIWIDTGIHSR
          YHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRPAIWIDTGIHSR
          EWITHATGIWTANKIVSDYGKDRVLTDILNAMDIFIELVTNPDGFAFTHSMNRLWRKNKS
          EW:T.A:G:W A.KI..DYG:D...T IL:.:DIF:E:VTNPDGFAFTHS.NR:WRK.:S
          EWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRS
          IRPGIFCIGVDLNRNWKSGFGGNGSNSNPCSETYHGPSPQSEPEVAAIVNFITAHGNFKA
           ..G .CIGVD NRNW.:GFG :G::SNPCSETYHG. ..SE EV.:IV:F:. HGN.KA
          HTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVKDHGNIKA
                                                          active site
          LISIHSYSQMLMYPYGRLLEPVSNQRELYDLAKDAVEALYKVHGIEYIFGSISTTLYVAS
          .ISIHSYSQ:LMYPYG  .EPV.:Q EL .L:K AV.AL .::G.:: :GSI ..:Y AS
```

FISI**H**SYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAI**Y**QAS

active site

GITVDWAYDSGIKYAFSF**E**LRDTGQYGFLLPATQIIPTAQETWMALRTIMEHTLNHPY 436

G T:DW.Y..GIKY:F:F**E**LRDTG:YGFLLPA:QIIPTA:ETW:AL TIMEHTLNHPY

GSTIDWTYSQGIKYSFTF**E**LRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHPY 419

Y: ACTIVE SITE, PROTON DONOR (BY SIMILARITY).

E: ACTIVE SITE, NUCLEOPHILE (BY SIMILARITY).

H, **E**, **H**: ZINC BINDING (BY SIMILARITY).

CIGVDLNRNWKSGFGGNGSNSNP**C**: DISULFID (BY SIMILARITY).

Fig. 10

BLASTP - alignment of 356 against pdb|1PCA|1PCA

Procarboxypeptidase a

This hit is scoring at : 5e-142 (expectation value)

Alignment length (overlap) : 399

Identities : 58 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Database searched : nrdb

```
Q:      36 NFTGDQVLRVLAKDEKQLSLLGDLEGLKPQKVDFWRGPARPSLPVDMRVPFSELKDIKAY
           :F.G.QVLR: ..DE.Q:. : :LE.L:  ::DFWRGPARP..P:D:RVPF..:: :K.:
H:       3 DFVGHQVLRISVDDEAQVQKVKELEDLEHLQLDFWRGPARPGFPIDVRVPFPSIQAVKVF
           LESHGLAYSIMIKDIQVLLDEERQAMAKSRRLERSTNSFSYSSYHTLEEIYSWIDNFVME
           LE:HG:.Y:IMI:D:Q:LLDEE::.M .S:  .R:T::F:Y::YHTLEEIY.::D .V.E
           LEAHGIRYTIMIEDVQLLLDEEQEQMFASQGRARTTSTFNYATYHTLEEIYDFMDILVAE
           HSDIVSKIQIGNSFENQSILVLKFSTGGSRHPAIWIDTGIHSREWITHATGIWTANKIVS
           H. :VSK:QIG.S:E.:.I.VLKFSTGGS..PAIWID:GIHSREWIT.A:G:W A.KI..
           HPALVSKLQIGRSYEGRPIYVLKFSTGGSNRPAIWIDSGIHSREWITQASGVWFAKKITE
           DYGKDRVLTDILNAMDIFIELVTNPDGFAFTHSMNRLWRKNKSIRPGIFCIGVDLNRNWK
           :YG::. .T IL::MDIF:E:VTNP:GFAFTHS NRLWRK.:S ..G .C:G D NRNW.
           NYGQNSSFTAILDSMDIFLEIVTNPNGFAFTHSDNRLWRKTRSKASGSLCVGSDSNRNWD
           SGFGGNGSNSNPCSETYHGPSPQSEPEVAAIVNFITAHGNFKALISIHSYSQMLMYPYGR
           :GFGG G::S:PC:ETYHG. P.SE EV.:I.:F:. :GN.KA.ISIHSYSQ:L:YPYG
           AGFGGAGASSSPCAETYHGKYPNSEVEVKSITDFVKNNGNIKAFISIHSYSQLLLYPYGY
           LLEPVSNQRELYDLAKDAVEALYKVHGIEYIFGSISTTLYVASGITVDWAYDSGIKYAFS
            .:. :::.EL .:AK.AV.AL .::G..Y :GSI T.:Y ASG .:DW.Y:.GIKY:FS
           KTQSPADKSELNQIAKSAVAALKSLYGTSYKYGSIITVIYQASGGVIDWTYNQGIKYSFS
           FELRDTGQYGFLLPATQIIPTAQETWMALRTIMEHTLNH     434
           FELRDTG: GFLLPA:QIIPTAQETW:AL TIMEHTLN:
           FELRDTGRRGFLLPASQIIPTAQETWLALLTIMEHTLNN     401
```

Fig. 11

Prosite Search

Access#	From->To	Name	Doc#
PS00132	187->210	CARBOXYPEPT_ZN_1	PDOC00123
PS00133	323->334	CARBOXYPEPT_ZN_2	PDOC00123

BLOCKS

AC#	Description	Strength	Score
BL00132B	Zinc carboxypeptidases, zinc-binding region 1	1300	1582
AA# 186 PAIWIDTGIHSREW			
BL00132E	Zinc carboxypeptidases, zinc-binding region 1	1338	1563
AA# 286 PCSETYHGPSPqSEPEVAAIVNFITaH			
BL00132C	Zinc carboxypeptidases, zinc-binding region 1	1359	1550
AA# 216 YGKDrvlTDILNAMDIFIElVTNPDGFAFTHSmNRLWRKNK			
BL00132F	Zinc carboxypeptidases, zinc-binding region 1	1338	1489
AA# 314 NFKALISIHSYSQMLMYPYGrl			
BL00132A	Zinc carboxypeptidases, zinc-binding region 1	1256	1412
AA# 138 YHTLEEIYSWIDNfVmEHsDIVSKIQIGNSFENqsIlVLKF			
BL00132D	Zinc carboxypeptidases, zinc-binding region 1	1221	1381
AA# 260 PGiFCIGVDLNRNWk			
BL00132G	Zinc carboxypeptidases, zinc-binding region 1	1213	1327
AA# 371 TTLYvASGiTVDWAYDSG			

Fig. 12

HMMPFAM - alignment of 356 against pfam|hmm|Zn_carbOpept

Zinc carboxypeptidase

This hit is scoring at : 417.0 E=1.7e-121

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    139 YHTLEEIYSWIDNFVMEHSDIVSKIQIGNSFENQSILVLKFSTG---GSRHPAIWIDTG-
          YH.LEEIY:W:D .V....D:VSK:.IG.S:E.:.: VLK.S..   G...P.::. .G
H:      1 YhnleeiyawlDllvsnfPdLvskvsiGksyeGRdlkvLKisdnpatgenePevfavagW

          IHSREWITHATGIWTANKIVSDYGKDRVLTDILNAMD-IFIELVTNPDGFAFTHSMN--R
          IH:REW:T.AT :W...::V::YG.D:.:T.:L:.:D .:I  V NPDG:A:: :.:  R
          iHAREwvtsAtllwllkelvanYgsDktitkllдgldlfyilpvfNpDGyaYsittdSyR

          LWRKNKSIRPGIFCIGVDLNRNWKSGFGGNGSNS-NPCSETYHGPSPQSEPEVAAIVNFI
          :WRK.:S ..G FC:G.D NRNW : :GG G::S :PCSETY.G.:P SEPE..A: :FI
          mWRKtRspnagsfcvGtDpNRNWyaqwggmgassysPcSetYeGtapfSepEtkavedfi

          TAHG-----NFKALISIHSYSQMLMYPYGRLLEPVSNQRELYDLA--KDAVEALYKVHGI
          .:       N.KA.I:.HSYSQ:L:YPYG  ..  .:...:L :L:  K A.:AL . HG.
          rswlgGGkqnIkayItfHsYSqlllyPYgydynlnpdandldelsdlkiaadalsarhgt

          EYIFG-SISTTLYVAS-GITVDWAYDSG-IKYAFSFELR-DTGQYG---FLLPATQIIPT
           Y..G . S:T:Y AS G : DWAYD G IKYAF:FELR DTG.YG   FLLP..QIIPT
          yYtlglpgsstIYpasAGGsdDwaydvgiikyaftfElrpdtgsyGnPCFllPeeqIipt

          AQ-E     419

          .. E

          gsee     304
```

Fig. 13

```
HMMPFAM - alignment of 356 against pfam|hmm|Propep_M14
Carboxypeptidase activation peptide
This hit is scoring at : 100.3   E=3.8e-26
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Q:      41 QVLRVLAKDEKQLSLLGDLEGLKPQKVDFWRG----PARPSLPVDMRVPFSELKDIKAYL
           QVLRV ..DE.Q:.LL DLE..:  ::DFW:     P.:P. .VD.RVP .::: :K::L
H:       1 qVlrvkvadedQvkllkdLentehleLDFWkpdsatpikpgstvDfrVpaediqavksfL
           ESHGLAYSIMIKDIQVLLDEER     118
           E..G: Y.::I:D:Q LL:E:
           eqsgIhYevlIeDVqelLeeqf      82
```

Fig. 14

```
BLASTP - alignment of 356 against trembl|M75106|HSPCPBX_1
gene: "pCPB"; Human prepro-plasma carboxypeptidase B mRNA, complete cds.
//:pironly|A41204|A41204 carboxypeptidase B (EC 3.4.17.2) CPB2 precursor -
human
//:gp|M75106|189687 gene: "pCPB"; Human prepro-plasma carboxypeptidase B
mRNA,
complete cds.

This hit is scoring at : 2e-70 (expectation value)
Alignment length (overlap) : 406
Identities : 38 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

Q:      37 FTGDQVLRVLAKDEKQLSLLGDLEGLKPQKVDFWRGPARPSLPVDMRVPF----SELKDI
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<150> 60/257,295

<151> 2000-12-26

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<170> PatentIn version 3.1

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Asp Glu Lys Gln Leu Ser Leu Leu Gly Asp Leu Glu Gly Leu Lys Pro
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Gln Lys Val Asp Phe Trp Arg Gly Pro Ala Arg Pro Ser Leu Pro Val
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Asp Met Arg Val Pro Phe Ser Glu Leu Lys Asp Ile Lys Ala Tyr Leu
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Glu Ser His Gly Leu Ala Tyr Ser Ile Met Ile Lys Asp Ile Gln Val
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Leu Leu Asp Glu Glu Arg Gln Ala Met Ala Lys Ser Arg Arg Leu Glu
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Arg Ser Thr Asn Ser Phe Ser Tyr Ser Ser Tyr His Thr Leu Glu Glu
     130                 135                 140

Ile Tyr Ser Trp Ile Asp Asn Phe Val Met Glu His Ser Asp Ile Val
145                 150                 155                 160

Ser Lys Ile Gln Ile Gly Asn Ser Phe Glu Asn Gln Ser Ile Leu Val
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Leu Lys Phe Ser Thr Gly Gly Ser Arg His Pro Ala Ile Trp Ile Asp
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Thr Gly Ile His Ser Arg Glu Trp Ile Thr His Ala Thr Gly Ile Trp
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Thr Ala Asn Lys Ile Val Ser Asp Tyr Gly Lys Asp Arg Val Leu Thr
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Lys Ser Ile Arg Pro Gly Ile Phe Cys Ile Gly Val Asp Leu Asn Arg
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Asn Trp Lys Ser Gly Phe Gly Gly Asn Gly Ser Asn Ser Asn Pro Cys
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Ser Glu Thr Tyr His Gly Pro Ser Pro Gln Ser Glu Pro Glu Val Ala
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Ala Ile Val Asn Phe Ile Thr Ala His Gly Asn Phe Lys Ala Leu Ile
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Ala Val Glu Ala Leu Tyr Lys Val His Gly Ile Glu Tyr Ile Phe Gly
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Ser Ile Ser Thr Thr Leu Tyr Val Ala Ser Gly Ile Thr Val Asp Trp
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Ala Tyr Asp Ser Gly Ile Lys Tyr Ala Phe Ser Phe Glu Leu Arg Asp
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Leu Asp Glu Glu Gln Glu Gln Met Phe Ala Phe Arg Ser Arg Ala Arg
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Ser Thr Asp Thr Phe Asn Tyr Ala Thr Tyr His Thr Leu Glu Glu Ile
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Tyr Asp Phe Leu Asp Leu Leu Val Ala Glu Asn Pro His Leu Val Ser
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Lys Ile Gln Ile Gly Asn Thr Tyr Glu Gly Arg Pro Ile Tyr Val Leu
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Lys Phe Ser Thr Gly Gly Ser Lys Arg Pro Ala Ile Trp Ile Asp Thr
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Gly Ile His Ser Arg Glu Trp Val Thr Gln Ala Ser Gly Val Trp Phe
            180                 185                 190

Ala Lys Lys Ile Thr Gln Asp Tyr Gly Gln Asp Ala Ala Phe Thr Ala
        195                 200                 205

Ile Leu Asp Thr Leu Asp Ile Phe Leu Glu Ile Val Thr Asn Pro Asp
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Gly Phe Ala Phe Thr His Ser Thr Asn Arg Met Trp Arg Lys Thr Arg
225                 230                 235                 240

Ser His Thr Ala Gly Ser Leu Cys Ile Gly Val Asp Pro Asn Arg Asn
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Trp Asp Ala Gly Phe Gly Leu Ser Gly Ala Ser Ser Asn Pro Cys Ser
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Glu Thr Tyr His Gly Lys Phe Ala Asn Ser Glu Val Glu Val Lys Ser
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Ile Val Asp Phe Val Lys Asp His Gly Asn Ile Lys Ala Phe Ile Ser
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Ile His Ser Tyr Ser Gln Leu Leu Met Tyr Pro Tyr Gly Tyr Lys Thr
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325 330 335

Val Thr Ala Leu Ala Ser Leu Tyr Gly Thr Lys Phe Asn Tyr Gly Ser
340 345 350

Ile Ile Lys Ala Ile Tyr Gln Ala Ser Gly Ser Thr Ile Asp Trp Thr
355 360 365

Tyr Ser Gln Gly Ile Lys Tyr Ser Phe Thr Phe Glu Leu Arg Asp Thr
370 375 380

Gly Arg Tyr Gly Phe Leu Leu Pro Ala Ser Gln Ile Ile Pro Thr Ala
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