AGGRECANASE MOLECULES

RELATED APPLICATION

[001] This application relies on the benefit of priority of U.S. provisional patent application Nos. 60/303,051, filed on July 5, 2001, and 60/349,133, filed January 16, 2002.

FIELD OF THE INVENTION

[002] The present invention relates to the discovery of nucleotide sequences encoding novel aggrecanase molecules, the aggrecanase proteins and processes for producing them. The invention further relates to the development of inhibitors of, as well as antibodies to the aggrecanase enzymes. These inhibitors and antibodies may be useful for the treatment of various aggrecanase-associated conditions including osteoarthritis.

BACKGROUND OF THE INVENTION

[003] Aggrecan is a major extracellular component of articular cartilage. It is a proteoglycan responsible for providing cartilage with its mechanical properties of compressibility and elasticity. The loss of aggrecan has been implicated in the degradation of articular cartilage in arthritic diseases. Osteoarthritis is a debilitating disease which affects at least 30 million Americans (MacLean *et al.*, *J Rheumatol* 25:2213-8 (1998)). Osteoarthritis can severely reduce quality of life due to degradation of articular cartilage and the resulting chronic pain. An early and important characteristic of the osteoarthritic process is loss of aggrecan from the extracellular matrix (Brandt and Mankin, *Pathogenesis of Osteoarthritis*, in Textbook of Rheumatology, WB Saunders Company, Philadelphia, PA, at 1355-1373

(1993)). The large, sugar-containing portion of aggrecan is thereby lost from the extra-cellular matrix, resulting in deficiencies in the biomechanical characteristics of the cartilage.

[004] A proteolytic activity termed "aggrecanase" is thought to be responsible for the cleavage of aggrecan thereby having a role in cartilage degradation associated with osteoarthritis and inflammatory joint disease. Work has been conducted to identify the enzyme responsible for the degradation of aggrecan in human osteoarthritic cartilage. Two enzymatic cleavage sites have been identified within the interglobular domain of aggrecan. One (Asn^{341}-Phe^{342}) is observed to be cleaved by several known metalloproteases. Flannery *et al.*, *J Biol Chem* 267:1008-14 (1992); Fosang *et al., Biochemical J.* 304:347-351 (1994). The aggrecan fragment found in human synovial fluid, and generated by IL-1 induced cartilage aggrecan cleavage is at the Glu^{373}-Ala^{374} bond (Sandy *et al.*, *J Clin Invest* 69:1512-1516 (1992); Lohmander *et al.,* Arthritis Rheum 36: 1214-1222 (1993); Sandy *et al., J Biol Chem* 266: 8683-8685 (1991)), indicating that none of the known enzymes are responsible for aggrecan cleavage *in vivo*.

[005] Recently, identification of two enzymes, aggrecanase-1 (ADAMTS 4) and aggrecanase-2 (ADAMTS-11) within the "Disintegrin-like and Metalloprotease with Thrombospondin type 1 motif" (ADAM-TS) family have been identified which are synthesized by IL-1 stimulated cartilage and cleave aggrecan at the appropriate site (Tortorella *et al.*, *Science* 284:1664-6 (1999); Abbaszade *et al., J Biol Chem* 274: 23443-23450 (1999)). It is

possible that these enzymes could be synthesized by osteoarthritic human articular cartilage. It is also contemplated that there are other, related enzymes in the ADAM-TS family which are capable of cleaving aggrecan at the Glu^{373}-Ala^{374} bond and could contribute to aggrecan cleavage in osteoarthritis. There is a need to identify other aggrecanase enzymes and determine ways to block their activity.

SUMMARY OF THE INVENTION

[006] The present invention is directed to the identification of novel aggrecanase protein molecules capable of cleaving aggrecan, the nucleotide sequences which encode the aggrecanase enzymes, and processes for the production of aggrecanases. These enzymes are contemplated to be characterized as having proteolytic aggrecanase activity. The invention further includes compositions comprising these enzymes.

[007] The invention also includes antibodies to these enzymes, in one embodiment, for example, antibodies that block aggrecanase activity. In addition, the invention includes methods for developing inhibitors of aggrecanase which block the enzyme's proteolytic activity. These inhibitors and antibodies may be used in various assays and therapies for treatment of conditions characterized by the degradation of articular cartilage.

[008] The invention provides an isolated DNA molecule comprising a DNA sequence chosen from: the sequence of SEQ ID NO. 5 from nucleotide #1-#2270; SEQ ID NO. 7 from nucleotide #1-#2339; SEQ ID NO. 3 from nucleotide #1 to #3899; SEQ ID NO. 9 from nucleotide #1 to #5004; SEQ. ID

NO. 11 from nucleotide #1 to #3369; and naturally occurring human allelic sequences and equivalent degenerative codon sequences.

[009] The invention also comprises a purified aggrecanase protein comprising an amino acid sequence chosen from: the amino acid sequence set forth in SEQ ID NO. 6 from amino acid #1-#756; SEQ ID NO. 8 from amino acid #1-#779; Figure 2 (SEQ ID NO. 10) from amino acid #1-#1057; Figure 5 (SEQ ID NO. 13) from amino acid #1-#1122; and homologous aggrecanase proteins consisting of addition, substitution, and deletion mutants of the sequences.

[010] The invention also provides a method for producing a purified aggrecanase protein produced by the steps of culturing a host cell transformed with a DNA molecule according to the invention, and recovering and purifying from said culture medium a protein comprising the amino acid sequence set forth in one of SEQ. ID NOs. 6, 8, 10, and 13.

[011] The invention also provides an antibody that binds to a purified aggrecanase protein of the invention. It also provides a method for developing inhibitors of aggrecanase comprising the use of aggrecanase protein chosen from SEQ ID NOs. 6 8, 10, 13, and a fragment thereof.

[012] Additionally, it provides a pharmaceutical composition for inhibiting the proteolytic activity of aggrecanase, wherein the composition comprises at least one antibody according to the invention and at least one pharmaceutical carrier. It also provides a method for inhibiting aggrecanase in a mammal comprising administering to said mammal an effective amount of

the pharmaceutical composition and allowing the composition to inhibit aggrecanase activity.

BRIEF DESCRIPTION OF THE DRAWINGS

[013] Figure 1 is the nucleotide sequence of an aggrecanase protein as set forth in SEQ ID NO. 9.

[014] Figure 2 is the amino acid sequence (SEQ ID NO. 10) of an aggrecanase protein encoded from the nucleotide sequence as set forth in SEQ ID NO. 9.

[015] Figure 3 is an extended nucleotide sequence (SEQ ID NO. 11) of EST14.

[016] Figure 4 is an exon insert of 69 bases (SEQ ID NO. 12) from nucleotide #2138(7) through #2206(7) for SEQ ID NO. 11.

[017] Figure 5 is the predicted protein translation (SEQ ID NO. 13) of SEQ ID NO. 11.

[018] Figure 6 is an amino acid sequence (SEQ ID NO. 14) containing SEQ ID NO. 5 and 24 extra in frame amino acids as a result of an additional exon.

BRIEF DESCRIPTION OF THE SEQUENCES

a.a. = amino acid

SEQUENCES	FIGURES	DESCRIPTION
1		EST 14
2		a.a. seq. of EST 14
3		aggrecanase DNA
4		a.a. seq. of SEQ ID NO. 3
5		aggrecanase DNA
6		a.a. seq. of SEQ ID NO. 5
7		aggrecanase DNA
8		a.a. seq. of SEQ ID NO. 7
9	Figure 1	aggrecanase DNA
10	Figure 2	a.a. seq. of SEQ ID NO. 9
11	Figure 3	aggrecanase DNA
12	Figure 4	exon nucleotide insert
13	Figure 5	a.a. seq. of SEQ ID NO. 11
14	Figure 6	exon a.a. insert
15		zinc binding signature region of aggrecanase-1
16		nucleotide insert
17		nucleotide sequence containing an insert with an Xho1 site
18		a 68 bp adapter nucleotide sequence
19		exon nucleotide insert
20		exon a.a. insert
21		primer
22		primer
23		primer
24		primer
25		primer
26		primer
27		primer
28		primer
29		primer
30		primer
31		synthesized nucleotides

32		synthesized nucleotides
33		synthesized nucleotides
34		synthesized nucleotides

DETAILED DESCRIPTION OF THE INVENTION

I. Novel Aggrecanase Proteins

[019] In one embodiment, the nucleotide sequence of an aggrecanase molecule of the present invention is set forth in SEQ ID NO. 3, as nucleotides #1 to #3899. It is contemplated that nucleotides #80-134 represent the pro domain. The metalloprotease domain comprises nucleotides #135-#254; intron nucleotides #255-#317, nucleotides #318-#560, intron nucleotides #561-#1264, nucleotides #1265-#1372, intron nucleotides #1373-#1801, and nucleotides #1802-#1976. The disintegrin domain comprises nucleotides #1977-#2236. The thrombospondin type I domain comprises amino acids #2237-#2492. The spacer region comprises amino acids #2493-#2636, intron nucleotides #2637-#2759, and nucleotides #2760-#3233. The thrombospondin type I sub motif comprises nucleotides #3234-#3416. The invention further includes equivalent degenerative codon sequences of the sequence set forth in SEQ ID NO. 3, as well as fragments thereof which exhibit aggrecanase activity. The full length sequence of the aggrecanase of the present invention may be obtained using the sequences of SEQ ID NO. 3 to design probes for screening for the full sequence using standard techniques.

[020] The amino acid sequence of the isolated aggrecanase-like molecule is set forth in SEQ ID. NO. 4, as nucleotides #1 to #807. The partial

Pro domain comprises amino acids #1-#18. A probable PACE processing site comprises amino acids #15-#18. The proposed metalloprotease domain comprises amino acids #19-#209. A partial catalytic Zn binding domain comprises amino acids #145-#155. The Met turn is amino acid #168. The proposed disintegrin domain comprises amino acids #210-#298. The proposed thrombospondin type I domain comprises amino acids #299-#377. The proposed cysteine rich and cysteine poor spacer domain comprises amino acids #378-#586. The proposed thrombospondin type I sub motif comprises amino acids #587-#644. Amino acids #648-#807 are an intron

[020] sequence. The invention further includes fragments of the amino acid sequence which encode molecules exhibiting aggrecanase activity.

[021] In another embodiment, the nucleotide sequence of an aggrecanase molecule of the present invention derived from thymus DNA is set forth in SEQ ID NO. 5 from nucleotide #1-#2270. The invention includes longer aggrecanase sequences obtained using the sequences of SEQ ID NO. 5 to design probes for screening. The invention further includes equivalent degenerative codon sequences of the sequence set forth in SEQ ID NO. 5, as well as fragments thereof which exhibit aggrecanase activity.

[022] The nucleotide sequence of the thymus clones set forth in SEQ ID NO. 5 encodes the amino acid sequence set forth in SEQ ID NO. 6 from amino acid #1-#756. With respect to SEQ ID NO. 6 the domains are contemplated as follows: The pro-domain comprises amino acid #1-#88. The probable PACE site is represented by amino acids RERR, amino acids #85-

#88. The metalloprotease domain comprises amino acids #89-#317 with catalytic Zn binding domain at #264-265, and a Met turn at #278. The disintegrin domain comprises amino acids #318-#408. The thrombospondin type I domain comprises amino acids #409-#487. The cysteine rich and cysteine poor spacer domain comprises amino acids #488-#695. The proposed thrombospondin type I sub motif comprises amino acids #696-#752. The invention further includes fragments of the amino acid sequence set forth in SEQ ID NO. 6 which encode molecules exhibiting aggrecanase activity.

[023] In a further embodiment, the nucleotide sequence of an aggrecanase molecule of the present invention derived from liver DNA is set forth in SEQ ID NO. 7 from nucleotide #1-#2339. The invention includes longer aggrecanase sequences obtained using the sequences of SEQ ID NO. 7 to design probes for screening. The invention further includes equivalent degenerative codon sequences of the sequence set forth in SEQ ID NO. 7, as well as fragments thereof which exhibit aggrecanase activity. The invention further includes fragments of the amino acid sequence set forth in SEQ ID NO. 8 which encode molecules exhibiting aggrecanase activity.

[024] The nucleotide sequence set forth in SEQ ID NO. 7 encodes the amino acid sequence set forth in SEQ ID NO. 8 from amino acid #1-#779. This sequence contains a 69 base insertion encoding from amino acid #578-#601 found in the spacer domain. The domains are contemplated as follows: The pro-domain comprises amino acid #1-#88. The probable PACE site is represented by amino acids RERR , amino acids #85-#88. The

metalloprotease domain comprises amino acids #89-#317 with catalytic Zn binding domain at #264-265, and a Met turn at #278. The disintegrin domain comprises amino acids #318-#408. The thrombospondin type I domain comprises amino acids #409-#487. The cysteine rich and cysteine poor spacer domain comprises amino acids #488-#577 and #602-#718. The proposed thrombospondin type I sub motif comprises amino acids #719-#776.

[025] In a further embodiment, the nucleotide sequence of an aggrecanase molecule of the present invention is set forth in SEQ ID NO. 9 from nucleotide #1-#5004. The invention further includes equivalent degenerative codon sequences of the sequence set forth in SEQ ID NO. 9, as well as fragments thereof which exhibit aggrecanase activity.

[026] The nucleotide sequence set forth in SEQ ID NO. 9 encodes the amino acid sequence set forth in SEQ ID NO. 10 from amino acid #1-#1057. The Pro domain is contemplated to comprise amino acids #1(R) through #158(R) (probable PACE processing site is underlined in Figure 2). The proposed metalloprotease domain comprises amino acids 159 (N) through 378 (K) with catalytic Zn binding domain at #324-335, Met turn at #347. The proposed disintegrin domain comprises amino acid #379 (V) through #478 (D). The proposed thrombospondin type I domain comprises amino acid #479 (G) through #557 (L). The proposed cysteine rich and cysteine poor spacer domain comprises amino acids #558 (L) through #760 (Q). The proposed thrombospondin type I sub motifs (4) comprise amino acids #761 (D) through #990 (C). The proposed PLAC domain comprises amino acids #991(N)

through #1057 (S) (found in C terminus of papilin, lacunin, PACE4 and PC5/6 proteases as well as ADAMTS2, ADAMTS3, ADAMTS10, ADAMTS12 and EST16). The invention further includes fragments of the amino acid sequence set forth in SEQ ID NO. 10 which encode molecules exhibiting aggrecanase activity.

[027] In a further embodiment, the nucleotide sequence of an aggrecanase molecule of the present invention is set forth in SEQ ID NO. 11 from nucleotide #1-#3369. The invention further includes equivalent degenerative codon sequences of the sequence set forth in SEQ ID NO. 11, as well as fragments thereof which exhibit aggrecanase activity.

[028] The nucleotide sequence set forth in SEQ ID NO. 11 encodes the amino acid sequence set forth in SEQ ID NO. 13 from amino acid #1-#1122. The proposed leader sequence comprises amino acids #1(M) through #21 (G). The proposed Pro domain comprises amino acids #22 (L) through #223 (R) (probable PACE processing site is underlined in Figure 5). Amino acid #244 (M) is the proposed first met of N-terminal alternate splice variant. The proposed metalloprotease domain comprises amino acids #224 (N) through #443 (K) with catalytic Zn binding domain at #389-400, and a Met turn at #413. The proposed disintegrin domain comprises amino acids #444(V) through #543(D). The proposed thrombospondin type I domain comprises amino acids #544(G) through #522. The proposed cysteine rich and cysteine poor spacer domain comprises amino acids #523(L) to #830(I). The proposed thrombospondin type I sub motifs (4) comprises amino acids #831(W) to

#1055(C). The proposed PLAC domain comprises amino acids #1056 (N) through #1022(S). NxS/Tx proposed N-linked glycosylation comprise amino acids #167-169 (NNS), #812-814 (NRT) #817-819 (NQS), amino acids #859-861 (NKT), amino acids #866-868 (NDS) and amino acids #921-923 (NGT). The invention further includes fragments of the amino acid sequence set forth in SEQ ID NO. 13 which encode molecules exhibiting aggrecanase activity.

[029] The invention includes methods for obtaining the full length aggrecanase molecule, the DNA sequence obtained by this method and the protein encoded thereby. The method for isolation of the full length sequence involves utilizing the aggrecanase sequence set forth in SEQ ID NOs. 3, 5, 7, 9, and 11 to design probes for screening, or otherwise screen, using standard procedures known to those skilled in the art. The preferred sequence for designing probes is the longer sequence of SEQ ID NOs. 5 or 7.

[030] The human aggrecanase protein or a fragment thereof may be produced by culturing a cell transformed with a DNA sequence chosen from SEQ ID NOs. 3, 5, 7, 9, and 11 and recovering and purifying from the culture medium a protein characterized by an amino acid sequence set forth in at least one of SEQ ID NOs. 4, 6, 8, 10, and 13 substantially free from other proteinaceous materials with which it is co-produced. For production in mammalian cells, the DNA sequence further comprises a DNA sequence encoding a suitable propeptide 5' to and linked in frame to the nucleotide sequence encoding the aggrecanase enzyme.

[031] The human aggrecanase proteins produced by the method discussed above are characterized by having the ability to cleave aggrecan and having an amino acid sequence chosen from SEQ ID NOs. 4, 6 , 8, 10, or 13 variants of the amino acid sequence of SEQ ID NOs. 4, 6 , 8, 10, or 13 including naturally occurring allelic variants, and other variants in which the proteins retain the ability to cleave aggrecan characteristic of aggrecanase proteins. Preferred proteins include a protein which is at least about 80% homologous, and more preferably at least about 90% homologous, to the amino acid sequence shown in SEQ ID NOs. 4, 6, 8, 10, or 13. Finally, allelic or other variations of the sequences of SEQ ID NOs. 4, 6, 8 , 10, or 13 whether such amino acid changes are induced by mutagenesis, chemical alteration, or by alteration of DNA sequence used to produce the protein, where the peptide sequence still has aggrecanase activity, are also included in the present invention. The present invention also includes fragments of the amino acid sequence of SEQ ID NOs. 4, 6, 8 , 10, or 13 which retain the activity of aggrecanase protein.

II. Identification of Homologous Aggrecanase Proteins and DNA Encoding Them

[032] It is expected that additional human sequences and other species have DNA sequences homologous to human aggrecanase enzymes. The invention, therefore, includes methods for obtaining the DNA sequences encoding other aggrecanase proteins, the DNA sequences obtained by those methods, and the protein encoded by those DNA sequences. This method entails utilizing the nucleotide sequence of the invention or portions thereof to

design probes to screen libraries for the corresponding gene from other species or coding sequences or fragments thereof from using standard techniques. Thus, the present invention may include DNA sequences from other species, which are homologous to the human aggrecanase protein and can be obtained using the human sequence. The present invention may also include functional fragments of the aggrecanase protein, and DNA sequences encoding such functional fragments, as well as functional fragments of other related proteins. The ability of such a fragment to function is determinable by assay of the protein in the biological assays described for the assay of the aggrecanase protein.

[033] For example, the amino acid translation of SEQ ID NO. 20 was used in a query against the databases TREMBL, swissprot, NCBI NR, PIR, and geneseqp in a BLASTP 2.2.2 search. Several sequences were identified as similar to SEQ ID NO. 20, differing only by splicing or incomplete sequence. These sequences were identified by the following accession numbers: AAE10350, AAE10347, AAU72894, AAE10349, AAE10348. It is believed that these sequences are all part of the same family of ADAMTS. One member of this family has already been published as ADAMTS17, which appears to have as its nearest family member ADAMTS19. The cloning of ADAMTS17 has been described in Cal, S., *et al.*, *Gene*, 283 (1-2), 49-62 (2002).

[034] SEQ ID NO. 11 was used as a query against the genesqn database using BLASTN 2.2.2. SEQ ID NO. 11 was determined to have

identity (with variable splicing or incomplete sequence) to several published sequences. For example, the published sequences were cited in EP-A2-1134286 (AAD17498, AAD17499, AAD17500, AAD17501, and AAD17502) and WO 20/0183782 (AAS97177).

[035] Some examples of homologous, non-human sequences include a mouse sequence 20834206 (found in the NCBI NR database), a rat sequence 13242316 (found in the NCBI NR database), a worm sequence AAY53898 (found in the geneseqp1 database), and a cow sequence 11131272 (found in the NCBI NR database). It is expected that these sequences, from non-human species, are homologous to human aggrecanase enzymes.

[036] The aggrecanase proteins provided herein also include factors encoded by the sequences similar to those of SEQ ID NOs. 3, 5, 7, 9 or 11, but into which modifications or deletions are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the protein) or deliberately engineered. For example, synthetic proteins may wholly or partially duplicate continuous sequences of the amino acid residues of SEQ ID NOs. 4, 6, 8, 10, or 13. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with aggrecanase proteins may possess biological properties in common therewith. It is known, for example that numerous conservative amino acid substitutions are possible without significantly modifying the structure and conformation of a protein, thus maintaining the biological

properties as well. For example, it is recognized that conservative amino acid substitutions may be made among amino acids with basic side chains, such as lysine (Lys or K), arginine (Arg or R) and histidine (His or H); amino acids with acidic side chains, such as aspartic acid (Asp or D) and glutamic acid (Glu or E); amino acids with uncharged polar side chains, such as asparagine (Asn or N), glutamine (Gln or Q), serine (Ser or S), threonine (Thr or T), and tyrosine (Tyr or Y); and amino acids with nonpolar side chains, such as alanine (Ala or A), glycine (Gly or G), valine (Val or V), leucine (Leu or L), isoleucine (Ile or I), proline (Pro or P), phenylalanine (Phe or F), methionine (Met or M), tryptophan (Trp or W) and cysteine (Cys or C). Thus, these modifications and deletions of the native aggrecanase may be employed as biologically active substitutes for naturally-occurring aggrecanase and in the development of inhibitors or other proteins in therapeutic processes. It can be readily determined whether a given variant of aggrecanase maintains the biological activity of aggrecanase by subjecting both aggrecanase and the variant of aggrecanase, as well as inhibitors thereof, to the assays described in the examples.

[037] Other specific mutations of the sequences of aggrecanase proteins described herein involve modifications of glycosylation sites. These modifications may involve O-linked or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at asparagine-linked glycosylation recognition sites. The asparagine-linked glycosylation recognition sites

comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Additionally, bacterial expression of aggrecanase-related protein will also result in production of a non-glycosylated protein, even if the glycosylation sites are left unmodified.

III. Novel Aggrecanase Nucleotide Sequences

[038] Still a further aspect of the invention are DNA sequences coding for expression of an aggrecanase protein having aggrecanase proteolytic activity or other disclosed activities of aggrecanase. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in SEQ ID NOs. 3, 5, 7, 9 and 11 and DNA sequences which, but for the degeneracy of the genetic code, are identical to the DNA sequence of SEQ ID NOs. 3, 5, 7, 9 and 11 and encode an aggrecanase protein.

[039] Further included in the present invention are DNA sequences which hybridize under stringent conditions with the DNA sequence of SEQ ID NOs. 1, 3, 5, 7, 9 and 11 and encode a protein having the ability to cleave aggrecan. Preferred DNA sequences include those which hybridize under stringent conditions (*see* Maniatis *et al*, *Molecular Cloning (A Laboratory Manual)*, Cold Spring Harbor Laboratory, at 387-389 (1982)). Such stringent conditions comprise, for example, 0.1X SSC, 0.1% SDS, at 65°C. It is

generally preferred that such DNA sequences encode a protein which is at least about 80% homologous, and more preferably at least about 90% homologous, to the sequence of set forth in SEQ ID NOs. 3, 5, 7, 9 or 11. Finally, allelic or other variations of the sequences of SEQ ID NOs. 1, 3, 5, 7, 9 or 11 whether such nucleotide changes result in changes in the peptide sequence or not, but where the peptide sequence still has aggrecanase activity, are also included in the present invention. The present invention also includes fragments of the DNA sequence shown in SEQ ID NOs 1, 3, 5, 7, 9 or 11 which encode a protein which retains the activity of aggrecanase.

[040] Similarly, DNA sequences which code for aggrecanase proteins coded for by the sequences of SEQ ID NO. 3, 5, 7, 9 or 11 or aggrecanase proteins which comprise the amino acid sequence of SEQ ID NOs. 4, 6, 8, 10, or 13 but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel factors described herein. Variations in the DNA sequences of SEQ ID NOs. 3, 5, 7, 9 or 11 which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the proteins encoded are also encompassed in the invention.

[041] The DNA sequences of the present invention are useful, for example, as probes for the detection of mRNA encoding aggrecanase in a given cell population. Thus, the present invention includes methods of

detecting or diagnosing genetic disorders involving the aggrecanase, or disorders involving cellular, organ or tissue disorders in which aggrecanase is irregularly transcribed or expressed. Antisense DNA sequences may also be useful for preparing vectors for gene therapy applications. Antisense DNA sequences are also useful for *in vivo* methods, such as to introduce the antisense DNA into the cell, to study the interaction of the antisense DNA with the native sequences, and to test the capacity of a promoter operatively linked to the antisense DNA in a vector by studying the interaction of antisense DNA in the cell as a measure of how much antisense DNA was produced.

[042] A further aspect of the invention includes vectors comprising a DNA sequence as described above in operative association with an expression control sequence therefor. These vectors may be employed in a novel process for producing an aggrecanase protein of the invention in which a cell line transformed with a DNA sequence encoding an aggrecanase protein in operative association with an expression control sequence therefor, is cultured in a suitable culture medium and an aggrecanase protein is recovered and purified therefrom. This process may employ a number of known cells both prokaryotic and eukaryotic as host cells for expression of the protein. The vectors may be used in gene therapy applications. In such use, the vectors may be transfected into the cells of a patient *ex vivo*, and the cells may be reintroduced into a patient. Alternatively, the vectors may be introduced into a patient *in vivo* through targeted transfection.

IV. Production of Aggrecanase Proteins

[043] Another aspect of the present invention provides a method for producing novel aggrecanase proteins. The method of the present invention involves culturing a suitable cell line, which has been transformed with a DNA sequence encoding an aggrecanase protein of the invention, under the control of known regulatory sequences. The transformed host cells are cultured and the aggrecanase proteins recovered and purified from the culture medium. The purified proteins are substantially free from other proteins with which they are co-produced as well as from other contaminants. The recovered purified protein is contemplated to exhibit proteolytic aggrecanase activity cleaving aggrecan. Thus, the proteins of the invention may be further characterized by the ability to demonstrate aggrecanase proteolytic activity in an assay which determines the presence of an aggrecan-degrading molecule. These assays or the development thereof is within the knowledge of one skilled in the art. Such assays may involve contacting an aggrecan substrate with the aggrecanase molecule and monitoring the production of aggrecan fragments (*see* for example, Hughes *et al.*, *Biochem J* 305: 799-804 (1995); Mercuri *et al*, *J Bio Chem* 274:32387-32395 (1999)).

[044] Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening, product production and purification are known in the art. (*See, e.g.*, Gething and Sambrook, *Nature*, 293:620-625 (1981); Kaufman et al, *Mol Cell Biol*, 5(7):1750-1759 (1985); Howley et al, U.S. Patent 4,419,446.) Another

suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable.

[045] Bacterial cells may also be suitable hosts. For example, the various strains of *E. coli* (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis, Pseudomonas*, other bacilli and the like may also be employed in this method. For expression of the protein in bacterial cells, DNA encoding the propeptide of aggrecanase is generally not necessary.

[046] Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the proteins of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. *See, e.g.,* Miller *et al.*, *Genetic Engineering*, 8:277-298 (Plenum Press 1986).

[047] Another aspect of the present invention provides vectors for use in the method of expression of these novel aggrecanase proteins. Preferably the vectors contain the full novel DNA sequences described above which encode the novel factors of the invention. Additionally, the vectors contain appropriate expression control sequences permitting expression of the aggrecanase protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present invention. Additionally, the sequence of SEQ ID NOs. 3, 5, 7, 9 or 11 or other sequences encoding aggrecanase proteins could be manipulated to express

composite aggrecanase proteins. Thus, the present invention includes chimeric DNA molecules encoding an aggrecanase protein comprising a fragment from SEQ ID NOs. 3, 5, 7, 9 or 11 linked in correct reading frame to a DNA sequence encoding another aggrecanase protein.

[048] The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Regulatory sequences for such vectors are known to those skilled in the art and may be selected depending upon the host cells. Such selection is routine and does not form part of the present invention.

V. Generation of Antibodies

[049] The purified proteins of the present inventions may be used to generate antibodies, either monoclonal or polyclonal, to aggrecanase and/or other aggrecanase-related proteins, using methods that are known in the art of antibody production. Thus, the present invention also includes antibodies to aggrecanase or other related proteins. The antibodies include both those that block aggrecanase activity and those that do not. The antibodies may be useful for detection and/or purification of aggrecanase or related proteins, or for inhibiting or preventing the effects of aggrecanase. The aggrecanase of the invention or portions thereof may be utilized to prepare antibodies that specifically bind to aggrecanase.

[050] The term "antibody" as used herein, refers to an immunoglobulin or a part thereof, and encompasses any protein comprising an antigen binding

site regardless of the source, method of production, and characteristics. The term includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, DCR-grafted antibodies. It also includes, unless otherwise stated, antibody fragments such as Fab, $F(ab')_2$, Fv, scFv, Fd, dAb, and other antibody fragments which retain the antigen binding function.

[051] Antibodies can be made, for example, via traditional hybridoma techniques (Kohler and Milstein, *Nature* 256:495-499 (1975)), recombinant DNA methods (U.S. Pat. No. 4,816,567), or phage display techniques using antibody libraries (Clackson et al., *Nature* 352: 624-628 (1991); Marks et al, *J. Mol. Biol.* 222:581-597 (1991)). For various other antibody production techniques, *see* Antibodies: A Laboratory Manual, eds. Harlow et al., Cold Spring Harbor Laboratory (1988).

[052] An antibody "specifically" binds to at least one novel aggrecanase molecule of the present invention when the antibody will not show any significant binding to molecules other than at least one novel aggrecanase molecule. The term is also applicable where, *e.g.*, an antigen binding domain is specific for a particular epitope, which is carried by a number of antigens, in which case the specific binding member (the antibody) carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope. In this fashion it is possible that an antibody of the invention will bind to multiple novel aggrecanase proteins. Typically, the

binding is considered specific when the affinity constant K_a is higher than 10^8 M^{-1}. An antibody is said to "specifically bind" or "specifically react" to an antigen if, under appropriately selected conditions, such binding is not substantially inhibited, while at the same time non-specific binding is inhibited. Such conditions are well known in the art, and a skilled artisan using routine techniques can select appropriate conditions. The conditions are usually defined in terms of concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of non-related molecules (e.g., serum albumin, milk casein), etc.

[053] Proteins are known to have certain biochemical properties including sections which are hydrophobic and sections which are hydrophilic. The hydrophobic sections would most likely be located in the interior of the structure of the protein while the hydrophilic sections would most likely be located in the exterior of the structure of the protein. It is believed that the hydrophilic regions of a protein would then correspond to antigenic regions on the protein. The hydrophobicity of SEQ ID NO. 11 was determined using GCG PepPlot. The results indicated that the n-terminus was hydrophobic presumably because of a signal sequence.

VI. Development of Inhibitors

[054] Various conditions such as osteoarthritis are known to be characterized by degradation of aggrecan. Therefore, an aggrecanase protein of the present invention which cleaves aggrecan may be useful for the development of inhibitors of aggrecanase. The invention therefore provides compositions comprising an aggrecanase inhibitor. The inhibitors may be

developed using the aggrecanase in screening assays involving a mixture of aggrecan substrate with the inhibitor followed by exposure to aggrecan. Inhibitors can be screened using high throughput processes, such as by screening a library of inhibitors. Inhibitors can also be made using three-dimensional structural analysis and/or computer aided drug design. The compositions may be used in the treatment of osteoarthritis and other conditions exhibiting degradation of aggrecan.

[055] The method may entail the determination of binding sites based on the three dimensional structure of aggrecanase and aggrecan and developing a molecule reactive with the binding site. Candidate molecules are assayed for inhibitory activity. Additional standard methods for developing inhibitors of the aggrecanase molecule are known to those skilled in the art. Assays for the inhibitors involve contacting a mixture of aggrecan and the inhibitor with an aggrecanase molecule followed by measurement of the aggrecanase inhibition, for instance by detection and measurement of aggrecan fragments produced by cleavage at an aggrecanase susceptible site. Inhibitors may be proteins or small molecules.

VII. Administration

[056] Another aspect of the invention therefore provides pharmaceutical compositions containing a therapeutically effective amount of aggrecanase antibodies and/or inhibitors, in a pharmaceutically acceptable vehicle. Aggrecanase-mediated degradation of aggrecan in cartilage has been implicated in osteoarthritis and other inflammatory diseases. Therefore, these compositions of the invention may be used in the treatment of diseases

characterized by the degradation of aggrecan and/or an up regulation of aggrecanase. The compositions may be used in the treatment of these conditions or in the prevention thereof.

[057] The invention includes methods for treating patients suffering from conditions characterized by a degradation of aggrecan or preventing such conditions. These methods, according to the invention, entail administering to a patient needing such treatment, an effective amount of a composition comprising an aggrecanase antibody or inhibitor which inhibits the proteolytic activity of aggrecanase enzymes.

[058] The antibodies and inhibitors of the present invention are useful to prevent, diagnose, or treat various medical disorders in humans or animals. In one embodiment, the antibodies can be used to inhibit or reduce one or more activities associated with the aggrecanase protein, relative to an aggrecanase protein not bound by the same antibody. Most preferably, the antibodies and inhibitors inhibit or reduce one or more of the activities of aggrecanase relative to the aggrecanase that is not bound by an antibody. In certain embodiments, the activity of aggrecanase, when bound by one or more of the presently disclosed antibodies, is inhibited at least 50%, preferably at least 60, 62, 64, 66, 68, 70, 72, 72, 76, 78, 80, 82, 84, 86, or 88%, more preferably at least 90, 91, 92, 93, or 94%, and even more preferably at least 95% to 100% relative to an aggrecanase protein that is not bound by one or more of the presently disclosed antibodies.

[059] Generally, the compositions are administered so that antibodies/their binding fragments are given at a dose between 1 µg /kg and 20 mg/kg, 1 µg/kg and 10 mg/kg, 1 µg/kg and 1 mg/kg, 10 µg/kg and 1 mg/kg, 10 µg/kg and 100 µg/kg, 100 µg and 1 mg/kg, and 500 µg/kg and 1 mg/kg. Preferably, the antibodies are given as a bolus dose, to maximize the circulating levels of antibodies for the greatest length of time after the dose. Continuous infusion may also be used after the bolus dose.

[060] In another embodiment and for administration of inhibitors, such as proteins and small molecules, an effective amount of the inhibitor is a dosage which is useful to reduce the activity of aggrecanase to achieve a desired biological outcome. Generally, appropriate therapeutic dosages for administering an inhibitor may range from 5 mg to 100 mg, from 15 mg to 85 mg, from 30 mg to 70 mg, or from 40 mg to 60 mg. Inhibitors can be administered in one dose, or at intervals such as once daily, once weekly, and once monthly. Dosage schedules can be adjusted depending on the affinity for the inhibitor to the aggrecanase target, the half-life of the inhibitor, and the severity of the patient's condition. Generally, inhibitors are administered as a bolus dose, to maximize the circulating levels of inhibitor. Continuous infusions may also be used after the bolus dose.

[061] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the

population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Antibodies and inhibitors, which exhibit large therapeutic indices, are preferred.

[062] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any antibody and inhibitor used in the present invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test antibody which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Levels in plasma may be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. Examples of suitable bioassays include DNA replication assays, transcription-based assays, GDF protein/receptor binding assays, creatine kinase assays, assays based on the differentiation of pre-adipocytes, assays based on glucose uptake in adipocytes, and immunological assays.

[063] The therapeutic methods of the invention include administering the aggrecanase inhibitor compositions topically, systemically, or locally as an implant or device. The dosage regimen will be determined by the attending

physician considering various factors which modify the action of the aggrecanase protein, the site of pathology, the severity of disease, the patient's age, sex, and diet, the severity of any inflammation, time of administration and other clinical factors. Generally, systemic or injectable administration will be initiated at a dose which is minimally effective, and the dose will be increased over a preselected time course until a positive effect is observed. Subsequently, incremental increases in dosage will be made limiting such incremental increases to such levels that produce a corresponding increase in effect, while taking into account any adverse affects that may appear. The addition of other known factors, to the final composition, may also affect the dosage.

[064] Progress can be monitored by periodic assessment of disease progression. The progress can be monitored, for example, by x-rays, MRI or other imaging modalities, synovial fluid analysis, patient perception, and/or clinical examination.

VIII. Assays and Methods of Detection

[065] The inhibitors and antibodies of the invention can be used in assays and methods of detection to determine the presence or absence of, or quantify aggrecanase in a sample. The inhibitors and antibodies of the present invention may be used to detect aggrecanase proteins, *in vivo* or *in vitro*. By correlating the presence or level of these proteins with a medical condition, one of skill in the art can diagnose the associated medical condition or determine its severity. The medical conditions that may be

diagnosed by the presently disclosed inhibitors and antibodies are set forth above.

[066] Such detection methods for use with antibodies are well known in the art and include ELISA, radioimmunoassay, immunoblot, western blot, immunofluorescence, immuno-precipitation, and other comparable techniques. The antibodies may further be provided in a diagnostic kit that incorporates one or more of these techniques to detect a protein (e.g., an aggrecanase protein). Such a kit may contain other components, packaging, instructions, or other material to aid the detection of the protein and use of the kit. When protein inhibitors are used in such assays, protein-protein interaction assays can be used.

[067] Where the antibodies and inhibitors are intended for diagnostic purposes, it may be desirable to modify them, for example, with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme). If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms, electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase can be detected by its ability to convert tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. Other suitable binding partners include biotin and avidin

or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art.

EXAMPLES

EXAMPLE 1: Isolation of DNA

[068] Potential novel aggrecanase family members were identified using a database screening approach. Aggrecanase-1 (*Science* 284:1664-1666 (1999)) has at least six domains: signal, propeptide, catalytic domain, disintegrin, tsp and c-terminal. The catalytic domain contains a zinc binding signature region, TAAHELGHVKF (SEQ. ID NO. 15) and a "MET turn" which are responsible for protease activity. Substitutions within the zinc binding region in the number of the positions still allow protease activity, but the histidine (H) and glutamic acid (E) residues must be present. The thrombospondin domain of Aggrecanase-1 is also a critical domain for substrate recognition and cleavage. It is these two domains that determine our classification of a novel aggrecanase family member. The protein sequence of the Aggrecanase-1 DNA sequence was used to query against the GeneBank ESTs focusing on human ESTs using TBLASTN. The resulting sequences were the starting point in the effort to identify full length sequence for potential family members. The nucleotide sequence of the aggrecanase of the present invention is comprised of an EST that contains homology over the catalytic domain and zinc binding motif of Aggrecanase-1. EST14 (SEQ ID NO. 1), a compilation of three ESTs (GenBank accession AW575922, AW501874, AW341169) was used to predict a peptide, SEQ ID NO. 2, having similarity to a portion of the Pro and Catalytic domains of

ADAMTS4. In SEQ ID NO. 1, bases #20-#581 are most homologous to ADAMTS 7 with a 37% identity. The predicted translation of nucleotides #21-#581 encodes part of the Pro domain (bases #21-#317); PACE processing site; and partial metalloprotease domain (bases #318-#581). EST14 was located on the human genome (Celera Discovery System (Rockville, MD, USA) and Celera's associated databases) and precomputed gene predictions (FgenesH) were used to extend EST14 sequence as shown in SEQ ID NO. 3. It is contemplated to be truncated by 600-700 bases and the C terminus is expected to be truncated.

[069] The gene for EST14 was isolated using a PCR strategy with tissue sources initially determined by preliminary PCR. Using 5' primer sequence CCGGCTCCCTCGTCTCGCTCAG (SEQ ID NO. 21) and 3' primer sequence AGCAGAAGGGCTGGGGGTCAAGGAC (SEQ ID NO. 22) on nine different Marathon-Ready cDNAs from Clontech (Palo Alto, CA, USA), a172 bp fragment corresponding to nucleotide # 52-224 of SEQ ID NO. 1 was generated using the Advantage-GC2 PCR kit from Clontech. Reaction conditions were those recommended in the user manual and included 0.5ng cDNA and 20pmole of each primer per 50 μl reaction. Cycling conditions were as follows: 94°C for 1 min, one cycle; followed by 35 cycles consisting of 94°C for 30 sec/68°C for 3 min; followed by one cycle of 68°C for 3 min.

[070] To initiate cloning of EST14, a 2270 bp fragment (SEQ ID NO. 5) or a 2339 bp fragment (SEQ ID NO. 7) encoding the middle portion of EST14 beginning at nucleotide #52 of the EST compilation in SEQ ID NO. 3

to nucleotide # 3416 of EST14 FgenesH prediction in SEQ ID NO. 3 were generated using 5' primer sequence CCGGCTCCCTCGTCTCGCTCAG (SEQ ID NO. 21)and 3' primer sequence ACGTGACTGGCAGGGGTGCAAGTT (SEQ ID NO. 23) from human thymus (pooled from 4 male and 1 female Caucasians) (SEQ ID NO. 5) or human liver (1 male Caucasian) (SEQ ID NO. 7) Marathon-Ready (from Clontech) cDNA substrates. The MasterAmp High Fidelity Extra-Long PCR kit from Epicentre Technologies (Madison, WI, USA) was used for the PCR reactions. Premix 4 or 8 were used as described in the user manual with 0.5ng cDNA and 20pmole of each primer per 50 μl reaction. Cycling conditions were as follows: 94°C for 3 min, one cycle; followed by 35 cycles consisting of 94°C for 30 sec/68°C for 4 min and; followed by cycle of 68°C for 6 min. The PCR products resulting from these amplifications were ligated into the pT-Adv vector using the AdvanTAge PCR Cloning Kit per manufacturer's instructions (Clontech). Ligated products were transformed into ElectroMAX DH5α-E cells from Invitrogen (Carlsbad, CA, USA). Clones originating from both libraries were sequenced to determine fidelity. This fragment's location in the full-length clone (SEQ ID NO. 11) is between nucleotides # 404 and 2674. The 69 base insertion in SEQ ID NO. 7 (from liver tissue) is also present in pancreas, kidney, and liver, but not thymus, testis, or leukemia MOLT 4 cDNA.

[071] A full determination of EST14 tissue distribution was achieved by probing a Clontech Human Multiple Tissue Expression Array (MTE). A probe for the MTE was generated from a PCR product amplifying the C-

terminal end of EST14 using 5' primer sequence CGGAGCATGTGGACGGAGACTGGA (SEQ ID NO. 24) and 3' primer sequence ACGTGACTGGCAGGGGTGCAAGTT (SEQ ID NO. 23) (nucleotide #2236 to #3416 of EST14 FgenesH prediction in SEQ ID NO. 3) on human thymus Marathon-Ready cDNA. The MasterAmp High Fidelity Extra-Long PCR kit from Epicentre Technologies was used for the PCR reactions using premix 4 and standard conditions as described above.

[072] The PCR product resulting from this amplification was ligated into the pT-Adv vector using the AdvanTAge PCR Cloning Kit (from Clontech) and sequenced. A probe encoding only the spacer domain was obtained after digestion of the plasmid containing the PCR product with the restriction endonucleases Blp I and EcoR I (NEB)(nucleotide #1842 to #2410 of Figure 3) using conditions recommended by New England Biolabs (Beverly, MA, USA). The 568bp fragment was isolated using a 5% nondenaturing polyacrylamide gel using standard molecular biology techniques found in Maniatis's Molecular Cloning A Laboratory Manual. The fragment was electroeluted out of the gel slice using Sample Concentration Cups from Isco (Little Blue Tank). The purified spacer domain probe was radiolabelled using the Ready-To-Go DNA Labelling Beads (dCTP) from Amersham Pharmacia Biotech (Piscataway, NJ, USA) per the manufacturer's instructions. The radiolabelled fragment was purified away from primers and unincorporated radionucleotides using a Nick column from Amersham Pharmacia Biotech per the manufacturer's instructions and then used to probe the MTE.

Manufacturer's conditions for hybridization of the MTE using a radiolabelled cDNA probe were followed. EST14 was found to be expressed in the following tissues and cell lines: thymus, leukemia MOLT4 cell line, pancreas, kidney, fetal thymus, and liver. For cloning the remaining portions of EST14 Clontech Marathon-Ready cDNAs of the following cell lines or tissues were used: human thymus pooled from 4 male and 1 female Caucasians, human pancreas pooled from 6 male Caucasians and human leukemia, lymphoblastic MOLT-4 cell line ATCC#CRL1582.

[073] The C-terminal sequence of EST14 was determined by 3' RACE using the Clontech Marathon cDNA Amplification Kit and human thymus and leukemia, lymphoblastic MOLT-4 cell line Marathon-ready cDNAs as substrates. 3' RACE primers used were: GSP1-TCTGGCTCTCAAAGACTCGGGTAA (SEQ ID NO. 25) (nucleotide #1811 to 1834 in SEQ ID NO. 5) and GSP2- GCAGGCACAACTGTTCGCTATGT (SEQ ID NO. 26) (nucleotide #1887 to 1909 in SEQ ID NO. 5). The Advantage-GC2 PCR Kit from Clontech was used to set up nested RACE reactions following instructions in the user manual for the Marathon cDNA Amplification Kit: the amount of GC melt used was 5 μl/50μl reaction, and the amount of GSP oligos used was 0.2pmole/μl. GSP1 primer was used for the first round of PCR and GSP2 primer was used for the nested reactions. Information from the 3' RACE is found between nucleotide #2095 and 5004 in SEQ ID NO. 9 / Figure 1 and includes an frame termination codon (TGA) at nucleotide # 3172 to 3174.

[074] A C-terminal 1079 bp fragment of EST14 including the stop codon was generated using 5' primer sequence GCAGGCACAACTGTTCGCTATGT (SEQ ID NO. 26) (nucleotide #2095 to 2117 of SEQ ID NO. 9) and 3' primer sequence TCACGAGCTCGGCGGTGGC (SEQ ID NO. 27) (nucleotide #3156 to 3174, complement, of SEQ ID NO. 9) on human thymus, pancreas and leukemia, lymphoblastic MOLT-4 cell line Marathon-Ready cDNAs used in the RACE reactions. The MasterAmp High Fidelity Extra-Long PCR kit from Epicentre Technologies was used for the PCR reactions using Premix 4 and standard conditions described above. The PCR products resulting from these amplifications were ligated into the pT-Adv vector using the AdvanTAge PCR Cloning Kit per manufacturer's instructions (Clontech). Ligated products were transformed into ElectroMAX DH5α-E cells from Invitrogen. Clones originating from all three libraries were sequenced to determine fidelity. This fragment's location in the full-length clone (Figure 3) is between nucleotides # 2290 and 3369.

[075] The N-terminal sequence of EST14 was determined by 5' RACE using the Clontech Marathon cDNA Amplification Kit and human thymus and leukemia, lymphoblastic MOLT-4 cell line Marathon-ready cDNAs as substrates. 5' RACE primers used were; GSP1-TCGGCCACCACCAGGGTCTCCAC (SEQ ID NO. 28) (nucleotide # 297 to 319, complement, in SEQ ID NO. 5) and GSP2-GTTCCTCCGCTCCCGCCAGTCCC (SEQ ID NO. 29) (nucleotide #247 to

269, complement, in SEQ ID NO. 5). The Advantage-GC2 PCR Kit from Clontech was used to set up nested RACE reactions following instructions in the user manual for the Marathon cDNA Amplification Kit: the amount of GC melt used was 5 μl/50μl reaction, and the amount of GSP oligos used was 0.2pmole/μl. GSP1 primer was used for the first round of PCR and GSP2 primer was used for the nested reactions. Information from the 5' RACE including the initiator Methionine (ATG) is found between nucleotide # 1 and 672 in Figure. 3.

[076] A N-terminal 685 bp fragment of EST14 including the initiator Methionine was generated using 5' primer sequence GGTCCCGGGTACCATGTGTGAC (SEQ ID NO. 30) (nucleotide #1 to 9 of Figure. 3) and 3' primer sequence GTTCCTCCGCTCCCGCCAGTCCC (SEQ ID NO. 29) (nucleotide # 650 to 672, complement, of Figure. 3) on human thymus Marathon-Ready cDNA used in the RACE reactions. The Advantage-GC2 PCR kit from Clontech was used for the PCR reactions. Reaction conditions were those recommended in the user manual and included 0.5ng cDNA and 20pmole of each primer per 50 μl reaction. Cycling conditions were as follows; 94°C for 2 min, one cycle: followed by 35 cycles consisting of 94°C for 20 sec/68°C for 3 min; followed by one cycle of 68°C for 3 min.

[077] The PCR products resulting from these amplifications were ligated into the pPCR-Script AMP vector using the PCR-Script AMP Cloning Kit per manufacturer's instructions (Stratagene, La Jolla, CA, USA). Ligated

products were transformed into ElectroMAX DH5α-E cells from Invitrogen. Clones were sequenced to determine fidelity.

[078] Cloned PCR fragments of EST14 were sequenced to determine fidelity. The full-length sequence for EST14 was the consensus derived from the EST14 FgenesH sequence (SEQ ID NO. 3) and the PCR products generated for EST14 from the three Clontech Marathon cDNAs (SEQ ID NO. 5, 9, and Figure 3). A full-length version of EST14 was constructed by moving the PCR products of the three fragments with correct sequences from pT-Adv or pPCR-Script AMP vectors into Cos expression vector pEDasc1 as follows. Two duplexes encoding a vector Xbal site (TCTAGA) at the 5' end, optimized Kozac sequence (GCCGCCACC) upstream of the initiator Met (ATG), to the EST14 N-terminal ApaL I site (GTGCAC) were synthesized in the following oligonucleotides; 5'-CTAGAGCCGCCACCATGTGTGACGGCGCCCTGCTGCCTCCGCTCGTCCTGCCCGTGCTGCTGCTGCTGGT (SEQ ID NO. 31) and complementary oligo 5'-GTCCCCAAACCAGCAGCAGCAGCACGGGCAGGACGAGCGGAGGCAGCAGGGCGCCGTCACACATGGTGGCGGCT (SEQ ID NO. 32), 5'-TTGGGGACTGGACCCGGGCACAGCTGTCGGCGACGCGGCGGCCGACGTGGAGGTGGTGCTCCCGTGGCGGGTGCGCCCCGACGACG (SEQ ID NO. 33) complementary oligo 5'-TGCACGTCGTCGGGGCGCACCCGCCACGGGAGCACCACCTCCACGTCGGCCGCCGCGTCGCCGACAGCTGTGCCCGGGTCCA (SEQ ID NO. 34).

These duplexes were joined with the ApaL 1 - SgrA 1 fragment of the N-terminus of EST14, SgrA 1 -Bgl II fragment of the middle portion of EST14 and a Bgl2 - Spe I fragment containing the C-terminus and stop codon (TGA) of EST14.

[079] The aggrecanase nucleotide sequence of the invention can be used to design probes for further screening for full length clones containing the isolated sequence. For example, EST14 may be used to locate smaller ESTs isolated from a variety of cDNA libraries. Examples of such ESTs, including the genbank accession number and their library origins are as follows: AA884550-- Soares_testis_NHT; AI808729-- Soares_NFL_T_GBC_S1 (pooled from fetal lung NbHL19W, testis NHT, and B-cell NCI_CGAP_GCB1); AI871510-- NCI_CGAP_Brn25 (anaplastic oligodendroglioma from brain); AI937739-- NCI_CGAP_Brn25 (anaplastic oligodendroglioma from brain); AW293573-- NCI_CGAP_Sub4 (colon); AW341169-- NCI_CGAP_Lu24 (carcinoid lung); AW501874-- NIH_MGC_52 (lymph germinal center B cells); AW575922-- NIH_MGC_52 (lymph germinal center B cells); BF529318--NCI_CGAP_Brn67(anaplastic oligodendroglioma with 1p/19q loss); BI828046-- NIH_MGC_119 (medulla brain); and BQ053458-- NIH_MGC_106 (natural killer cells, cell line).

[080] The final nucleotide sequence of EST14 from the Met to stop codon is set forth in SEQ ID NO. 11. In alternate splice variants exon 2 is missing 371 nucleotides from nucleotide #79 to #449 set forth in SEQ ID NO. 11 (counting the exon with the initiator Met as exon 1) which throws the frame

off at the N-terminus so the initiator Met is not in frame with the remainder of the protein. M is the first met found in sequence of this alternate splice variant. As seen above, the leader sequence and pro domain are missing from this truncated form. An additional exon can be found in certain cDNAs (liver, pancreas, kidney) that encodes for 24 extra in frame amino acids set forth in SEQ ID NO. 14 from amino acid #113(V) to #136(C) following the cysteine rich spacer domain in liver but not thymus cDNA including 4 extra cysteines. These extra cysteines are not found in any of the ADAMTS family members.

[081] The expression profile from Human Multiple Tissue Expression Array and Multiple Tissue Northerns from Clontech is as follows: moderate expression is found in lymphoblastic leukemia molt4 cell line and thymus. Lower expression is found in pancreas, kidney, and fetal thymus. Weak but detectable expression is found in liver, salivary gland, fetal brain, lymph node, colorectal adenocarcinoma SW480 cell line, fetal lung, trachea, fetal spleen, and testis.

EXAMPLE 2: Expression of Aggrecanase

[082] In order to produce murine, human or other mammalian aggrecanase-related proteins, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts including insect host cell culture systems by conventional genetic engineering techniques. Expression systems for biologically active recombinant human aggrecanase are

contemplated to be stably transformed mammalian cells, insect, yeast or bacterial cells.

[083] One skilled in the art can construct mammalian expression vectors by employing a sequence comprising SEQ ID NOs. 3, 5, 7, 9, 11 or other DNA sequences encoding aggrecanase-related proteins or other modified sequences and known vectors, such as pCD (Okayama *et al.*, *Mol Cell Biol*, 2:161-170 (1982)), pJL3, pJL4 (Gough *et al.*, *EMBO J*, 4:645-653 (1985)) and pMT2 CXM.

[084] The mammalian expression vector pMT2 CXM is a derivative of p91023(b) (Wong *et al.*, *Science* 228:810-815 (1985)) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described (Kaufman, *Proc. Natl. Acad. Sci. USA* 82:689-693 (1985)) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in *E. coli*.

[085] Plasmid pMT2 CXM is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in

linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2 CXM is then constructed using loopout/in mutagenesis (Morinaga, *et al.*, *Biotechnology* 84: 636 (1984)). This removes bases 1075 to 1145 relative to the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence: 5' PO-CATGGGCAGCTCGAG-3' (SEQ. ID NO. 16) at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease Xho I. A derivative of pMT2CXM, termed pMT23, contains recognition sites for the restriction endonucleases PstI, Eco RI, SalI and XhoI. Plasmid pMT2 CXM and pMT23 DNA may be prepared by conventional methods.

[086] pEMC2β1 derived from pMT21 may also be suitable in practice of the invention. pMT21 is derived from pMT2 which is derived from pMT2-VWF. As described above EcoRI digestion excises the cDNA insert present in pMT-VWF, yielding pMT2 in linear form which can be ligated and used to transform *E. Coli* HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

[087] pMT21 is derived from pMT2 through the following two modifications. First, 76 bp of the 5' untranslated region of the DHFR cDNA including a stretch of 19 G residues from G/C tailing for cDNA cloning is deleted. In this process, a XhoI site is inserted to obtain the following sequence immediately upstream from DHFR:

5' -CTGCAGGCGAGCCTGAATTCCTCGAGCCATCATG-3' (SEQ. ID NO. 17)

PstI Eco RI XhoI

[088] Second, a unique ClaI site is introduced by digestion with EcoRV and XbaI, treatment with Klenow fragment of DNA polymerase I, and ligation to a ClaI linker (CATCGATG). This deletes a 250 bp segment from the adenovirus associated RNA (VAI) region but does not interfere with VAI RNA gene expression or function. pMT21 is digested with EcoRI and XhoI, and used to derive the vector pEMC2B1.

[089] A portion of the EMCV leader is obtained from pMT2-ECAT1 (S.K. Jung, et al, *J. Virol* 63:1651-1660 (1989)) by digestion with Eco RI and PstI, resulting in a 2752 bp fragment. This fragment is digested with TaqI yielding an Eco RI-TaqI fragment of 508 bp which is purified by electrophoresis on low melting agarose gel. A 68 bp adapter and its complementary strand are synthesized with a 5' TaqI protruding end and a 3' XhoI protruding end which has the following sequence:

5'-

CGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTT

TaqI

GAAAAACACGATTGC-3' (SEQ. ID NO. 18)

XhoI

[090] This sequence matches the EMC virus leader sequence from nucleotide 763 to 827. It also changes the ATG at position 10 within the EMC virus leader to an ATT and is followed by a XhoI site. A three way ligation of the pMT21 Eco RI-XhoI fragment, the EMC virus EcoRI-TaqI fragment, and

the 68 bp oligonucleotide adapter TaqI-XhoI adapter resulting in the vector pEMC2β1.

[091] This vector contains the SV40 origin of replication and enhancer, the adenovirus major late promoter, a cDNA copy of the majority of the adenovirus tripartite leader sequence, a small hybrid intervening sequence, an SV40 polyadenylation signal and the adenovirus VA I gene, DHFR and β-lactamase markers and an EMC sequence, in appropriate relationships to direct the high level expression of the desired cDNA in mammalian cells.

[092] The construction of vectors may involve modification of the aggrecanase-related DNA sequences. For instance, aggrecanase cDNA can be modified by removing the non-coding nucleotides on the 5' and 3' ends of the coding region. The deleted non-coding nucleotides may or may not be replaced by other sequences known to be beneficial for expression. These vectors are transformed into appropriate host cells for expression of aggrecanase-related proteins. Additionally, the sequence of SEQ ID NOs. 3, 5, 7, 9, 11 or other sequences encoding aggrecanase-related proteins can be manipulated to express a mature aggrecanase-related protein by deleting aggrecanase encoding propeptide sequences and replacing them with sequences encoding the complete propeptides of other aggrecanase proteins.

[093] One skilled in the art can manipulate the sequences of SEQ ID NOs. 3, 5, 7, 9, or 11 by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create

bacterial vectors for intracellular or extracellular expression by bacterial cells. For example, the coding sequences could be further manipulated (*e.g.*, ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified aggrecanase-related coding sequence could then be inserted into a known bacterial vector using procedures such as described in *Taniguchi et al.*, *Proc Natl Acad Sci USA*, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and an aggrecanase-related protein expressed thereby. For a strategy for producing extracellular expression of aggrecanase-related proteins in bacterial cells, *see, e.g.*, European patent application EPA 177,343.

[094] Similar manipulations can be performed for the construction of an insect vector (*see, e.g.* procedures described in published European patent application EPA 155,476) for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. (*See, e.g.*, procedures described in published PCT application WO86/00639 and European patent application EPA 123,289).

[095] A method for producing high levels of a aggrecanase-related protein of the invention in mammalian, bacterial, yeast or insect host cell systems may involve the construction of cells containing multiple copies of the heterologous aggrecanase-related gene. The heterologous gene is linked to an amplifiable marker, e.g., the dihydrofolate reductase (DHFR) gene for

which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, *J Mol Biol*, 159:601-629 (1982). This approach can be employed with a number of different cell types.

[096] For example, a plasmid containing a DNA sequence for an aggrecanase-related protein of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A)3 (Kaufman and Sharp, *Mol Cell Biol* 2:1304 (1982)) can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by various methods including calcium phosphate coprecipitation and transfection, electroporation or protoplast fusion. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (e.g. sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman *et al.*, Mol Cell Biol., 5:1750 (1983). Transformants are cloned, and biologically active aggrecanase expression is monitored by the assays described above. Aggrecanase protein expression should increase with increasing levels of MTX resistance. Aggrecanase proteins are characterized using standard techniques known in the art such as pulse labeling with ^{35}S methionine or cysteine and polyacrylamide gel electrophoresis. Similar procedures can be followed to produce other related aggrecanase-related proteins.

[097] In one example the aggrecanase gene of the present invention set forth in SEQ ID NO. 11 may be cloned into the expression vector pED6 (Kaufman *et al.*, *Nucleic Acid Res* 19:44885-4490 (1991)). COS and CHO DUKX B11 cells are transiently transfected with the aggrecanase sequence of the invention (+/- co-transfection of PACE on a separate pED6 plasmid) by lipofection (LF2000, Invitrogen). Duplicate transfections are performed for each gene of interest: (a) one for harvesting conditioned media for activity assay and (b) one for 35-S-methionine/cysteine metabolic labeling.

[098] On day one media is changed to DME(COS) or alpha(CHO) media + 1% heat-inactivated fetal calf serum+/- 100µg/ml heparin on wells(a) to be harvested for activity assay. After 48h (day 4), conditioned media is harvested for activity assay.

[099] On day 3, the duplicate wells (b) are changed to MEM (methionine-free/cysteine free) media + 1% heat-inactivated fetal calf serum +100µg/ml heparin + 100µCi/ml 35S-methioine/cysteine (Redivue Pro mix, Amersham). Following 6h incubation at 37°C, conditioned media is harvested and run on SDS-PAGE gels under reducing conditions. Proteins are visualized by autoradiography.

EXAMPLE 3: Biological Activity of Expressed Aggrecanase

[0100] To measure the biological activity of the expressed aggrecanase-related proteins obtained in Example 2 above, the proteins are recovered from the cell culture and purified by isolating the aggrecanase-related proteins from other proteinaceous materials with which they are co-produced as well as from other contaminants. Purification is carried out using

standard techniques known to those skilled in the art. The purified protein may be assayed in accordance with the following assays:

[0101] Assays specifically to determine if the protein is an enzyme capable of cleaving aggrecan at the aggrecanase cleavage site:

[0102] 1. Flourescent peptide assay: Expressed protein is incubated with a synthetic peptide which encompasses amino acids at the aggrecanase cleavage site of aggrecan. One side of the synthetic peptide has a flourophore and the other a quencher. Cleavage of the peptide separates the flourophore and quencher and elicits flourescence. From this assay it can be determined that the expressed protein can cleave aggrecan at the aggrecanase site, and relative flourescence tells the relative activity of the expressed protein.

[0103] 2. Neoepitope western: Expressed protein is incubated with intact aggrecan. After several biochemical manipulations of the resulting sample (dialysis, chondroitinase treatment, lyophilization and reconstitution) the sample is run on an SDS PAGE gel. The gel is incubated with an antibody that only recognizes a site on aggrecan exposed after aggrecanase cleavage. The gel is transferred to nitrocellulose and developed with a secondary antibody (called a western assay) to result in bands running at a molecular weight consistent with aggrecanase generated cleavage products of aggrecan. This assay tells the expressed protein cleaved native aggrecan at the aggrecanase cleavage site, and also tells the molecular weight of the

cleavage products. Relative density of the bands can give some idea of relative aggrecanase activity.

[0104] Assay to determine if an expressed protein can cleave aggrecan anywhere in the protein (not specific to the aggrecanase site):

[0105] 3. Aggrecan ELISA: Expressed protein is incubated with intact aggrecan which had been previously adhered to plastic wells. The wells are washed and then incubated with an antibody that detects aggrecan. The wells are developed with a secondary antibody. If there is the original amount of aggrecan remaining in the well, the antibody will densely stain the well. If aggrecan was digested off the plate by the expressed protein, the antibody will demonstrate reduced staining due to reduced aggrecan concentration. This assay tells whether an expressed protein is capable of cleaving aggrecan (anywhere in the protein, not only at the aggrecanase site) and can determine relative aggrecan cleaving.

[0106] Protein analysis of the purified proteins is conducted using standard techniques such as SDS-PAGE acrylamide (Laemmli, Nature 227:680 (1970)) stained with silver (Oakley, *et al.*, *Anal Biochem.* 105:361 (1980)) and by immunoblot (Towbin, *et al., Proc. Natl. Acad. Sci. USA* 76:4350 (1979)). Using the above described assays, expressed aggrecanase-related proteins are evaluated for their activity and useful aggrecanase-related molecules are identified.

EXAMPLE 4: Preparation of Antibodies

[0107] An antibody against a novel aggrecanase molecule is prepared. To develop an antibody capable of inhibiting aggrecanase activity, a group of

mice are immunized every two weeks with a novel aggrecanase protein mixed in Freunds complete adjuvant for the first two immunizations, and incomplete Freunds adjuvant thereafter. Throughout the immunization period, blood is sampled and tested for the presence of circulating antibodies. At week 9, an animal with circulating antibodies is selected, immunized for three consecutive days, and sacrificed. The spleen is removed and homogenized into cells. The spleen cells are fused to a myeloma fusion partner (line P3-x63-Ag8.653) using 50% PEG 1500 by an established procedure (Oi & Herzenberg, *Selected Methods in Cellular Immunology*, W. J. Freeman Co., San Francisco, CA, at 351 (1980)). The fused cells are plated into 96-well microtiter plates at a density of 2×10^5 cells/well. After 24 hours, the cells are subjected to HAT selection (Littlefield, *Science,* 145: 709 (1964)) effectively killing any unfused and unproductively fused myeloma cells.

[0108] Successfully fused hybridoma cells secreting anti-aggrecanase antibodies are identified by solid and solution phase ELISAs. Novel aggrecanase protein is prepared from CHO cells as described above and coated on polystyrene (for solid phase assays) or biotinylated (for a solution based assay). Neutralizing assays are also employed where aggrecan is coated on a polystyrene plate and biotin aggrecanase activity is inhibited by the addition of hybridoma supernatant. Results identify hybridomas expressing aggrecanase antibodies. These positive clones are cultured and expanded for further study. These cultures remain stable when expanded and cell lines are cloned by limiting dilution and cryopreserved.

[0109] From these cell cultures, a panel of antibodies is developed that specifically recognize aggrecanase proteins. Isotype of the antibodies is determined using a mouse immunoglobulin isotyping kit (Zymed™ Laboratories, Inc., San Francisco, CA).

EXAMPLE 5: Method of Detecting Level of Aggrecanase

[0110] The anti-aggrecanase antibody prepared according to Example 4 can be used to detect the level of aggrecanase in a sample The antibody can be used in an ELISA, for example, to identify the presence or absence, or quantify the amount of, aggrecanase in a sample. The antibody is labeled with a fluorescent tag. In general, the level of aggrecanase in a sample can be determined using any of the assays disclosed in Example 3.

EXAMPLE 6: Method of Treating a Patient

[0111] The antibody developed according to Example 4 can be administered to patients suffering from a disease or disorder related to the loss of aggrecan, or excess aggrecanase activity. Patients take the composition one time or at intervals, such as once daily, and the symptoms and signs of their disease or disorder improve. For example, loss of aggrecan would decrease or cease and degradation of articular cartilage would decrease or cease. Symptoms of osteoarthritis would be reduced or eliminated. This shows that the composition of the invention is useful for the treatment of diseases or disorders related to the loss of aggrecan, or excess aggrecanase activity. The antibodies can also be used with patients susceptible to osteoarthritis, such as those who have a family history or markers of the disease, but have not yet begun to suffer its effects.

Patient's Condition	Route of Administration	Dosage	Frequency	Predicted Results
Osteoarthritis	Subcutaneous	500 µg/kg	Daily	Decrease in symptoms
"	"	1 mg/kg	Weekly	"
"	Intramuscular	500 µg/kg	Daily	"
"	"	1 mg/kg	Weekly	"
"	Intravenous	500 µg/kg	Daily	"
"	"	1 mg/kg	Weekly	"
Family History of Osteoarthritis	Subcutaneous	500 µg/kg	Daily	Prevention of condition
"	Intramuscular	500 µg/kg	Daily	"
"	Intravenous	500 µg/kg	Daily	"

[0112] The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto. All of the documents cited in this application are incorporated by reference in their entirety. Additionally, all sequences cited in databases and all references disclosed are incorporated by reference in their entirety.

What is claimed is:

1. An isolated DNA molecule comprising a DNA sequence chosen from:

a) the sequence of SEQ ID NO. 5 from nucleotide #1-#2270 ;

b) the sequence of SEQ ID NO. 7 from nucleotide #1-#2339 ;

c) the sequence of SEQ ID NO. 3 from nucleotide #1 to #3899; and

d) the sequence of SEQ ID NO. 9 from nucleotide #1 to #5001;

e) the sequence of SEQ. ID NO. 11 from nucleotide #1 to #3369; and

f) naturally occurring human allelic sequences and equivalent degenerative codon sequences of (a) through (e).

2. A vector comprising a DNA molecule of claim 1 in operative association with an expression control sequence therefor.

3. A host cell transformed with the DNA sequence of claim 1.

4. A host cell transformed with a DNA sequence of claim 2.

5. A method for producing a purified human aggrecanase protein, said method comprising:

a) culturing a host cell transformed with a DNA molecule according to claim 1; and

b) recovering and purifying said aggrecanase protein from the culture medium.

6. The method of claim 5, wherein said host cell is an insect cell.

7. A purified aggrecanase protein comprising an amino acid sequence chosen from:

a) the amino acid sequence set forth in SEQ ID NO. 6 from amino acid #1-#756;

b) the amino acid sequence set forth in SEQ ID NO. 8 from amino acid #1-#779;

c) the amino acid sequence set forth in SEQ ID NO. 10 from amino acid #1-#1057;

d) the amino acid sequence set forth in SEQ ID NO. 13 from amino acid #1-#1122; and

e) homologous aggrecanase proteins consisting of addition, substitution, and deletion mutants of the sequences of (a) through (d).

8. A purified aggrecanase protein produced by the steps of

a) culturing a cell transformed with a DNA molecule according to claim 1; and

b) recovering and purifying from said culture medium a protein comprising an amino acid sequence chosen from SEQ. ID NO. 6, 8, 10, and 13.

9. An antibody that binds to a purified aggrecanase protein of claim 7.

10. The antibody of claim 9, wherein the antibody inhibits aggrecanase activity.

11. A method for identifying inhibitors of aggrecanase comprising a) providing an aggrecanase protein chosen from:

i) SEQ ID NO. 6 or a fragment thereof;

ii) SEQ ID NO. 8 or a fragment thereof;

iii) SEQ. ID NO. 10 or a fragment thereof; and

iv) SEQ. ID NO. 13 or a fragment thereof;

b) combining the aggrecanase with a potential inhibitor and

c) evaluating whether the potential inhibitor inhibits aggrecanase activity.

12. The method of claim 11 wherein the method comprises evaluating the aggrecanase protein is used in a three dimensional structural analysis prior to combining with the potential inhibitor.

13. The method of claim 11 wherein the method comprises evaluating the aggrecanase protein is used in a computer aided drug design prior to combining with the potential inhibitor.

14. A pharmaceutical composition for inhibiting the proteolytic activity of aggrecanase, wherein the composition comprises an antibody according to claim 9 and a pharmaceutical carrier.

15. A method for inhibiting aggrecanase in a mammal comprising administering to said mammal an effective amount of the composition of claim 14 and allowing the composition to inhibit aggrecanase activity.

16. The method of claim 15, wherein the composition is administered intravenously, subcutaneously, or intramuscularly.

17. The method of claim 15, wherein the composition is administered at a dosage of from 500 µg/kg to 1 mg/kg.